Plant Physiol. (1987) 85, 823–827
0032-0889/87/85/0823/05/$01.00/0

Purification and Partial Characterization of a Hydroxyproline-Rich Glycoprotein in a Graminaceous Monocot, *Zea mays*[1]

Received for publication May 27, 1987 and in revised form July 20, 1987

MARCIA KIELISZEWSKI AND DEREK T. A. LAMPORT*
Department of Energy Plant Research Laboratory, and Department of Biochemistry, Michigan State University, East Lansing, Michigan 48824

ABSTRACT

Graminaceous monocots generally contain low levels of hydroxyproline-rich Glycoproteins (HRGPs). As HRGPs are often at the cell surface, we used the intact cell elution technique (100 millimolar $AlCl_3$) to isolate soluble surface proteins from *Zea mays* cell suspension cultures. Further fractionation of the trichloroacetic acid-soluble eluate on the cation exchangers phospho-cellulose and BioRex-70 gave several retarded, hence presumably basic fractions, which also contained hydroxyproline (Hyp). One of these fractions yielded a pure HRGP after a final purification step involving Superose-6 gel filtration. As this HRGP was unusually rich in threonine, (25 mole%) we designated it as a threonine-hydroxyproline-rich glycoprotein (THRGP); it contained about 27% carbohydrate occurring exclusively as arabinosylated Hyp, predominantly as the monosaccharide (15%), and trisaccharide (25%) with 48% Hyp nonglycosylated—a characteristically graminaceous monocot profile. Amino acid analysis confirmed the basic character, and gave a low alanine content. Reaction with Yariv artificial antigen was negative. These characteristics show that the THRGP is not an arabinogalactan protein. On the other hand, antibodies raised against tomato extensin P1 cross-reacted significantly with the THRGP; this cross-reactivity and the above analytical data provide the best evidence to date for the presence of extensin in a graminaceous monocot.

Current widespread interest in structural HRGPs[2] stems from their localization in the extracellular matrix and their possible roles in the growth regulation (3, 7), stress response (5, 9, 17, 19, 22, 23) cell-cell recognition (6, 22) and reproductive physiology (6). Understandably, work has concentrated on the richest sources of HRGPs which are: dicotyledonous higher plants (14, 22) and volvocalean algae (21). Monocotyledonous plants, in particular the GMs, are generally hydroxyproline-poor (14) and their HRGPs have been virtually ignored, yet monocots represent a highly significant group of angiosperms, not least because they contain the cereals which sustain civilization.

We report here the first isolation and partial characterization of an HRGP from a graminaceous monocot, *Zea mays*. We also provide evidence that this HRGP may be an extensin, a class of HRGP well known as a major structural component of the dicot primary cell wall. This HRGP is unusually rich in threonine, and hence we refer to it as a THRGP.

MATERIALS AND METHODS

Suspension Cultures. We grew maize cell suspensions (variety 'Black Mexican', a gift from Dr. Tom Hodges, Purdue University), in 1 L flasks containing 500 ml Murashige and Skoog medium (20) (2 mg/L, 2,4-D) shaken at 120 rpm on a gyrotary shaker at 27° under 900 lux of constant fluorescent lighting, and subcultured every 11 d to an initial packed cell volume of 3%.

Cell Wall Preparation. We prepared maize cell walls by suspending the cells in distilled H_2O, cooling in an ice bath, and sonicating the cells 10 min followed by alternating H_2O washes and pelleting the walls by bench-top centrifugation until the walls were free of cell debris, judging by microscopic examination. Next, we boiled the walls 5 min in 5% SDS, removing the SDS via alternating acetone washes and pelleting in a bench-top centrifuge followed by water washes and lyophilization.

Hydroxyproline Assay. We determined hydroxyproline after hydrolysis (6 N HCl, 110°C, 18 h) by Kivirikko's method (12) which involves alkaline hypobromite oxidation and subsequent coupling with acidic Ehrlich's reagent and monitoring at 560 nm.

Hydroxyproline Arabinoside Profile. We determined Hyp arabinosides (16) after alkaline hydrolysis (0.44 N $Ba(OH)_2$ 18 h, 105°C) careful neutralization with concentrated H_2SO_4, followed by centrifugation and freeze-drying the supernatant fraction. We redissolved the lyophilate in distilled H_2O and applied 0.5 mL containing 100 to 200 μg hydroxyproline to a 75 × 0.6 cm column (H^+ form) of Technicon Chromobeads C washed with water and eluted with a 0 to 0.5 N HCl gradient, monitoring the post-column hydroxyproline assay reaction at 560 nm.

Preparation of Crude HRGP by Intact Cell Elution. We prepared batches of crude THRGP from 11-d cultures (575 ml/flask; 17 flasks/batch) harvested on a 2 L coarsely sintered funnel followed by a water wash and then gentle agitation of the cell pad (about 600 g fresh weight) in 1 L of 100 mM $AlCl_3$ (a nonplasmolyzing concentration) for 3 min before final suction. The eluate was reduced in volume to 100 ml at 32°C. After adding TCA (final concentration 10% w/v) to the concentrated eluate (18 h, 4°C), centrifugation (13,000g, 45 min, SS-34 rotor head) yielded a hydroxyproline-poor pellet (0.5% Hyp dw, discarded) and hydroxyproline-rich supernatant which was dialysed 36 h at 4°C and then freeze-dried. We designate this TCA-soluble fraction 'crude HRGP.'

Phospho-Cellulose Ion Exchange Chromatography. We dissolved crude HRGP (10 mg/ml) in 12 mM (pH 3.0) McIlvaine buffer (18), and applied a maximum of 30 mg to a Bio-Rad

[1] Supported by the United States Department of Agriculture Grant No. 86-CRCR-1-2014 and by the United States Department of Energy contract DE-AC02-76ERO-1338.

[2] Abbreviations: HRGP, hydroxyproline-rich glycoprotein; THRGP, threonine-hydroxyproline-rich glycoprotein; GM, graminaceous monocot; dw, dry weight; TEM, transmission electron microscopy; V_o, void volume; CWP, cell wall preparation; P1, glycosylated extensin type 1; dP1, deglycosylated extensin type 1; P2, glycosylated extensin type 2; dP2, deglycosylated extensin type 2; CP1, Cellex-P peak 1; CP2, Cellex-P peak 2; CP3, Cellex-P peak 3; Hyp, hydroxyproline.

Cellex-P phospho-cellulose column (8 mm i.d. × 100 mm) equilibrated with 12 mM McIlvaine buffer (pH 3.0). We eluted with a 3.0 to 6.8 pH gradient (in McIlvaine buffer) followed by a 0 to 1 M NaCl gradient in 15 mM (pH 6.8) McIlvaine buffer at a flow rate of 19 ml/h, monitoring the absorbancy at 220 nm.

BioRex-70 Ion Exchange Chromatography. We dissolved the Cellex-P Peak 2 in 2 ml 30 mM sodium phosphate buffer (pH 7.4) buffer and applied a maximum of 20 mg to a BioRex 70 (100–200 mesh) column (8 mm i.d. × 100 mm) equilibrated with 30 mM sodium phosphate buffer (pH 7.4), and eluted with a buffered 0 to 1 M NaCl gradient at a flow rate of 19 ml/h, monitoring the absorbancy at 220 nm.

Gel Permeation Chromatography. We injected 1 mg of semi-purified THRGP in 250 μl 200 mM (pH 7.0), 0.02% azide-sodium phosphate buffer onto a Pharmacia Superose-6 HPLC gel filtration column, and eluted at a flow rate of 14 ml/h, monitoring the absorbancy at 220 nm.

Amino Acid Analysis. We used a Pickering High Speed Na^+ cation exchange column (3 mm i.d. × 150 mm) in series with a BX-8 cation exchange column (3.7 mm i.d. × 70 mm, Benson Co.) eluted by Pickering buffers A, B, and C. Postcolumn fluorometric detection involved NaOCl oxidation and OPA coupling which allowed Hyp and Pro detection (24). Data capture was by an IBM 9000 computer with IBM CAPs software.

Sugar Analyses. We analyzed sugars as their alditol acetates (1) by GC using a 6 foot × 2 mm i.d. PEGS224 column programmed from 130 to 180 at 4°/min for neutral sugars and a 6 foot × 2 mm i.d. OV 275 column programmed from 130 to 230 at 2°/min for amino sugars using an SP4100 computing integrator for data capture.

SDS Gel Electrophoresis. (Based on Laemmli and Favre [13]). We loaded 2 to 30 μg of THRGP to the 'sepracomb' of commercially prepared Sepra-gel gradient (10–20% polyacrylamide, Separation Science, Inc.) We detected protein by silver staining (30).

TEM Sample Preparation. We prepared THRGP for TEM by following the methods of Tyler and Branton (27).

Precipitation with β-Glycosyl Yariv Antigen. We reacted 400 μg of THRGP with Yariv Antigen according to Jermyn and Yeow (10).

THRGP Reaction with Polyclonal Antibodies Raised Against Tomato Extensin Precursors. We determined cross-reactivities of antibodies raised against tomato extensin precursors (11) with the THRGP by ELISA (based on Ref 8). We coated each test well of 96 well polystyrene plates (Nunc, Thomas Scientific) with 0.2 μg antigen (THRGP, P1, P2, dP1, dP2) in 200 μL (pH 9.6), 50 mM $NaHCO_3$ buffer, for 15 h at 4°C; washed the plate once in H_2O and briefly dried it before blocking all remaining protein binding sites by addition of 200 μl 1% BSA in PBS (final pH 7.5), for 30 min at 37°C, followed by washing twice with H_2O and then drying.

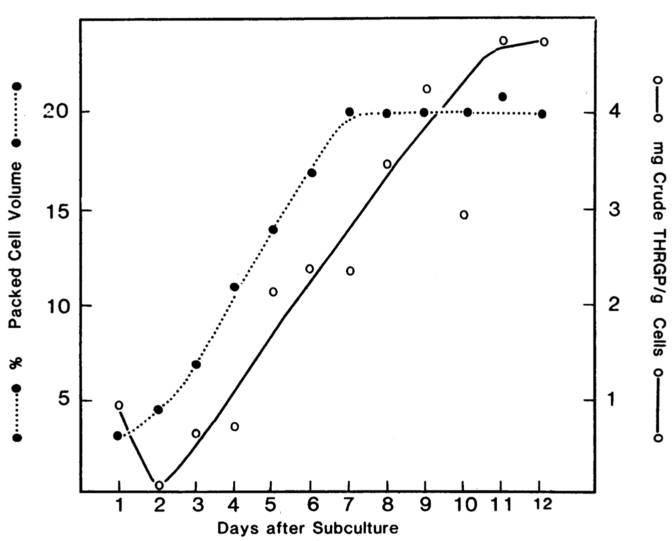

FIG. 1. Crude HRGP elution from the cell surface as a function of culture age. Total soluble eluate fell to a minimum at 2 d and rose to level off at d 11.

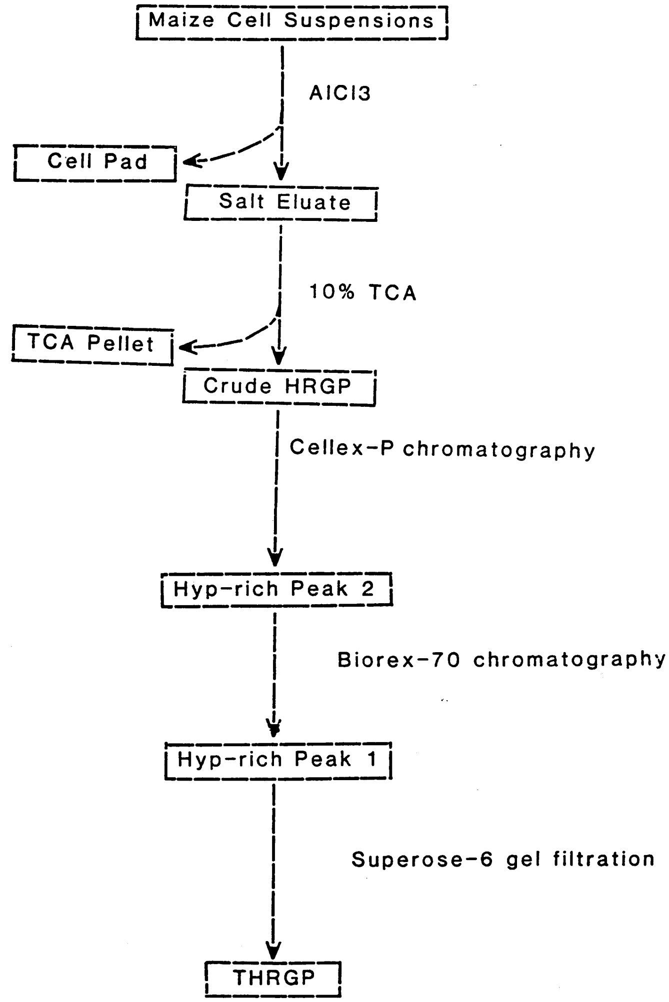

FIG. 2. THRGP purification flow chart. Fractionation of the crude eluate *via* cation exchange chromatography and gel filtration yielded pure THRGP.

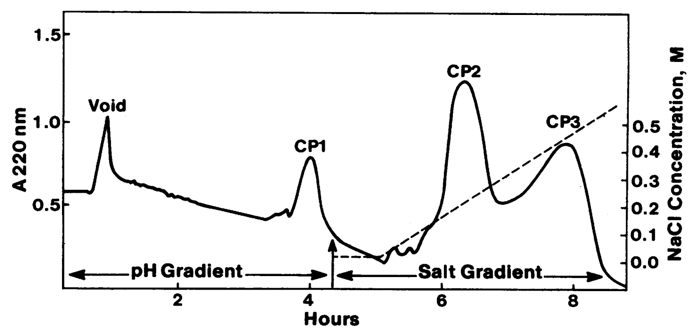

FIG. 3. Fractionation of the crude salt eluate from maize suspension cultured cells using cation exchange chromatography. A Cellex-P column was eluted with a citrate-phosphate pH gradient (pH 3.0–7.0) followed by a 0 to 1 M NaCl gradient in buffer. CP2 contains the THRGP.

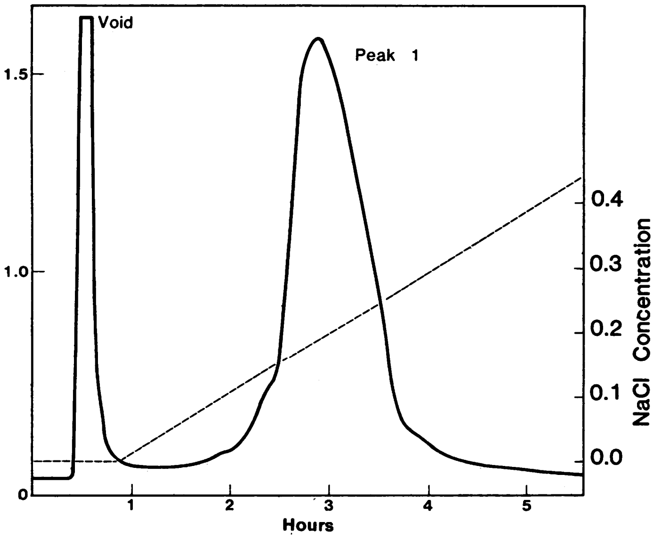

FIG. 4. Fractionation of Cellex-P peak 2 using weak cation-exchange chromatography. A BioRex-70 column was eluted with a 0 to 1 M NaCl gradient in sodium phosphate buffer. The THRGP is in peak 1.

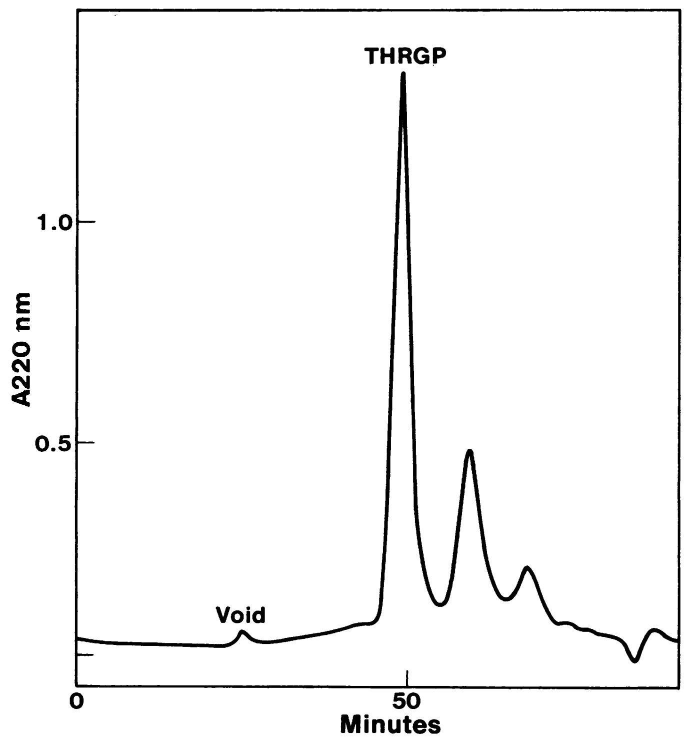

FIG. 5. Purification of THRGP using HPLC gel permeation chromatography. A Superose-6 column was eluted with 200 mM sodium phosphate buffer. The THRGP elutes at 48 min (2 × V_o).

We diluted the control (preimmune) and test sera as follows: ×200 for P1, dP1, and pre-immune control, and ×800 for P2, dP2, and preimmune control, in pH 7.5 PBS, and then added 25 µl of the diluted sera to the antigen-coated wells containing 25 µl 1% BSA-Tween-20 (1 µl/ml)/PBS at pH 7.5. After 1 h at 37°C we washed the plate twice in H_2O, added 50 µl diluted (×2000) goat-anti-rabbit serum coupled to peroxidase (Cappel Laboratories) in BSA/Tween-20/PBS to each well, incubated at 37°C for 30 min, washed the plate five times with H_2O, again briefly dried the plate, and then added 100 µl substrate to each well (11 mg ABTS and 15 µl 30% H_2O_2 in 50 ml pH 4, 50 mM citrate buffer). After 30 min incubation at 23°C we added 100 µl NaF/EDTA stopping reagent (prepared by adding 50 µl 40% w/v tetrasodium EDTA to 50 ml 6 mM NaF in 2.5 mM HF) to each well, and then determined the absorbance at 405 nm.

Assay of Agglutination. We assayed the agglutinating effect of a serial dilution of THRGP (100–10 ng/ml) on a 1% suspension of rabbit erythrocytes in phosphate-buffered saline according to the method of Allen and Neuberger (2).

RESULTS

THRGP Elution from the Cell Surface. The amount of elutable crude HRGPs rose as a function of culture age. After subculture, total soluble eluate fell to a minimum at 2 d and rose to level off at d 11 (Fig. 1). Thus, for bulk preparations, we used 11 d cells and 100 mM $AlCl_3$, the cells yielding 5.4 mg crude HRGP/g cells dw and fractionated further as shown in the flow sheet (Fig. 2). The crude HRGP was 1.7% Hyp on a dw basis (*i.e.* we recovered 92 µg soluble Hyp/g dw cells). The 100 mM $AlCl_3$ did not plasmolyse the cells.

Treatment of the Crude Eluate with 10% (w/v) TCA. Overnight precipitation with 10% (w/v) TCA at 4°C followed by centrifugation precipitated 50% by weight of the crude eluate. The TCA pellet was 72% protein and 0.5% Hyp dw, but the HRGP remained soluble. The yield of TCA-soluble crude HRGP was 2.7 mg/g cells dw. The crude HRGP was 60% protein and 3.5% Hyp dw, representing a 60-fold Hyp enrichment over the whole cell Hyp content of 0.06%.

Wall-Bound Protein and Hydroxyproline Content. The maize cell wall is about 12% protein, determined by amino acid analysis, and 0.15% Hyp dw, determined by the method of Kivirikko and Liesma (12).

Cation-Exchange Chromatography of the Dialysed TCA-Soluble Salt Eluate. Chromatography on phosphocellulose yielded a void and three major protein peaks, designated CP1, CP2, and CP3, respectively (Fig. 3). The void and CP1 contained a trace of Hyp while CP2 contained 8.4% Hyp dw, and CP3 contained 2.7% Hyp dw. CP1 eluted at pH 3.8 in the pH gradient, CP2 at 200 mM NaCl, and CP3 at 450 mM NaCl in the salt gradient. Further chromatography of CP2 on Biorex-70 (Fig. 4) gave a Hyp-poor void (4% Hyp dw) and a Hyp-rich THRGP fraction (12% Hyp dw), designated BioRex Peak 1, which eluted at 200 mM NaCl (Fig. 4).

Gel Filtration of BioRex Peak 1 THRGP. Gel filtration on Superose-6 gave a major Hyp-rich (18% Hyp dw), threonine-rich peak at 2 × V_o and a minor Hyp-containing peak (3% Hyp dw) at 2.5 × V_o (Fig. 5).

SDS Gel Electrophoresis of THRGP. The THRGP migrated as a single fuzzy band with an apparent mol wt of 71.6 kD.

Amino Acid Analysis of THRGP after Superose-6. The THRGP contained 25 mol% threonine, 24 mol% Hyp, and was rich in proline, lysine, and serine (Table I).

Neutral Sugar Analysis. Alditol acetate derivatization of the THRGP showed arabinose as the only THRGP sugar substituent. The arabinose:Hyp molar ratio was 1.44:1. The arabinose accounted for 27% by weight of the THRGP.

Hydroxyproline Arabinoside Profile. A Hyp-arabinoside profile of the THRGP showed 48% nonglycosylated Hyp and Hyp arabinoside 3 as the major glycosylated component (Table I). The arabinose accounted for 35% by weight of THRGP. A Hyp-arabinoside profile of the maize cell wall showed 24% nonglycosylated Hyp and Hyp tri-arabinoside as the major glycosylated component (Table I).

Electron Microscopy of THRGP. TEM shadowed preparations of the THRGP showed rod-like monomers (see "Discussion") averaging 70 ± 3 nm in length (Fig. 6).

Precipitation with β-Glycosyl Yariv Antigen. The THRGP did not react with Yariv Antigen, even at the relatively high level of 0.5 mg/ml where a standard AGP (0.5 mg/ml) (sycamore) gave

Table I. *THRGP Composition*

Amino Acid AA	Composition[a] at Mol%	Hyp Arabinoside Profile		
		Hyp arabinoside	THRGP % Hyp	Cell wall preparation[b] % Hyp
Hyp	24.8	1	15	9
Asp	0.7	2	6	6
Thr	25.3	3	25	41
Ser	7.3	4	6	20
Glu	2.3	Free Hyp	48	24
Pro	14.5	Total Hyp	100	100
Gly	2.4			
Ala	1.7			
Cys	0			
Val	0.7			
Met	0			
Ilu	0.1			
Leu	0.2			
Tyr	3.9			
Phe	0.1			
His	2.4			
Lys	13.5			
Arg	0.1			

[a] The average of amino acid analysis from three different THRGP preparations. [b] Prepared from maize cell suspension cultures.

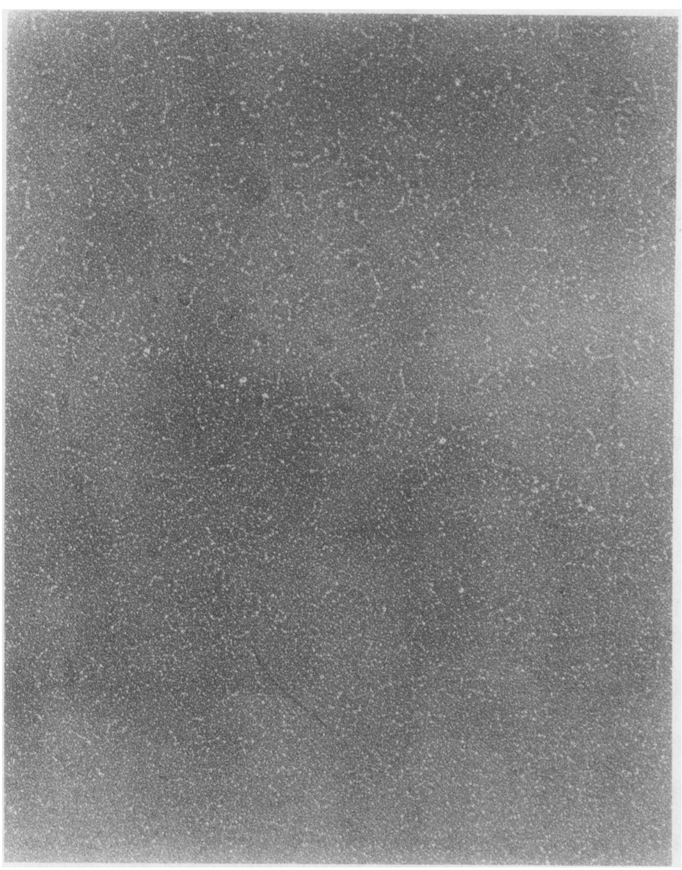

FIG. 6. TEM of the THRGP. TEM shadowed preparations of the maize THRGP appeared as rod-like monomers 70 nm long.

an A of 2.36 at 420 nm.

Reactivity of THRGP with Antibodies Raised Against Tomato Extensin Precursors as Determined by ELISA. Figure 7 shows the cross-reactivities of polyclonal antibodies with THRGP (we had previously raised these antibodies against tomato extensin precursors P1 and P2). Antibodies raised against glycosylated and deglycosylated tomato P2 (P2 and dP2) cross-reacted 28 and 12%, respectively, with glycosylated THRGP. Antibodies against P1 and deglycosylated P1 (P1 and dP1) cross-reacted 68 and 40%, respectively, with glycosylated THRGP. Control preimmune rabbit serum did not react with the THRGP.

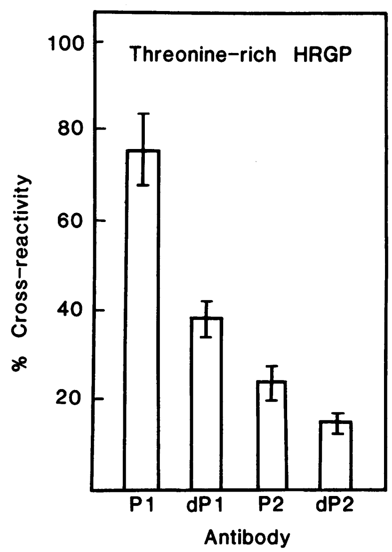

FIG. 7. Reactivity of THRGP with antibodies raised against tomato extensin precursors. Antibodies raised against glycosylated and deglycosylated tomato extensin precursor P1 (dP1) cross-reacted significantly with the THRGP. Antibodies raised against glycosylated and deglycosylated tomato precursor P2 (dP2) also cross-reacted with the THRGP, but to a lesser extent.

Assay of Agglutinin. THRGP did not agglutinate rabbit erythrocytes.

DISCUSSION

Monocots are, by comparison with dicots, relatively poor in Hyp, although it has been clear for some time that monocot

Hyp-containing proteins do exist, both in the grasses (4; A. Bleeker, H. Kende, unpublished data) and other monocot families. Thus, van Etten (28) identified the seedcoat as a tissue often enriched in Hyp, while early work showed that cell walls prepared from coleoptiles contained detectable Hyp (14) some of it O-arabinosylated although to a much less extent than in the dicots (16).

Much of this work implicitly assumes that easily soluble HRGPs correspond to arabinogalactan proteins, while the insoluble HRGPs correspond to extensin. The latter hypothesis is difficult to test. However, a recent reinvestigation of our 'intact cell elution' technique (14) showed that under optimal conditions, we could ionically desorb soluble monomeric extensin precursors to wall-bound extensin directly from the cell surface of intact tomato cells grown in suspension culture (24). We and others have characterized soluble extensin monomers, chemically (5, 25, 29), immunologically (11) and electron microscopically (26, 29), as a small family of highly glycosylated, basic, periodic, flexuous rodlike glycoproteins of about 80 nm contour length and about 50 nm persistence length. Thus, we now have the tools to determine whether or not extensin occurs in graminaceous monocots. The question is relevant to current ideas about the control of cell extension (*cf.* oat coleoptiles) and a recently proposed model for the primary cell wall of dicots (15), which invokes an extensin 'weft' to mechanically couple the load-bearing microfibrillar polymer 'warp'-cellulose.

Our data here show successful application of the intact cell elution technique to maize cell suspension cultures. More than 30 proteins appeared in the crude eluate but only four or five occurred as major components, of which at least two were HRGPs, one of them being unusually rich in threonine. We purified the THRGP to constant composition; it migrated as a single band on SDS-PAGE; however, the apparent size of 71.6 kD is probably an overestimate judging by its contour length and glycosylation profile. Its status as a monomer is suggested by its behavior on Superose-6 gel filtration and SDS gel electrophoresis. Ten amino acids accounted for 98 mol% of the amino acid residues, being richest in threonine and Hyp, each accounting for 25 mol%, with a high proline, lysine, serine content, and lesser but significant amounts of tyrosine, histidine, glycine, glutamate, alanine, and valine. Such a biased composition is typical of HRGPs in general and extensin in particular, although the threonine-rich feature is novel; and, like extensin, the THRGP is highly basic. Furthermore the total Hyp plus proline content of 39 mol% implies a polyproline-II conformation similar to extensin (29), while the Hyp arabinoside profile is consistent with an extensin glycosylation pattern, and corroborates earlier work (16) which showed a high (about 50%) proportion of nonglycosylated Hyp residues in the monocots. However, the absence of galactose from the THRGP as well as the high threonine content distinguish it from dicot extensins.

Transmission electron microscopy of low-angle rotary-shadowed material visualized THRGP molecules as flexuous rods similar to those of dicot extensins (26, 29) although somewhat shorter, but consistent with their elution position on Superose-6 close to dicot extensin monomers.

Polyclonal antibodies raised against tomato extensins P1 and P2 cross-reacted significantly with the THRGP confirming the presence of common epitopes and proclaiming these THRGP molecules at least 'extensin-like.' The extent of true homology awaits further peptide mapping (in progress) and amino acid sequence determination of the major repeating peptides if the THRGP is highly periodic like other extensins.

The intriguing question of THRGP role also awaits future work, but the rapid 'disappearance' of elutable THRGP within 48 h after subculture (Fig. 1), and its nondetectability in the growth medium, implies a covalent association with the primary cell wall, possibly as a network like the dicots, but as a very much looser weave.

Acknowledgments—We thank Pat Muldoon for amino acid analyses, Virginia Boone for medium preparation, Dr. John Heckman for the electron microscopy, and Dr. Jack Preiss for some very good advice.

LITERATURE CITED

1. Albersheim P, DJ Nevins, PD English, A Karr 1967 A method for the analysis of sugars in plant cell-wall polysaccharides by gas liquid chromatography. Carbohydr Res 5: 340–345
2. Allen AK, A Neuberger 1973 The purification and properties of the lectin from potato tubers, a hydroxyproline-containing glycoprotein. Biochem J 135: 307–314
3. Basile DV, MR Basile 1982 Evidence for a regulatory role of cell surface hydroxyproline-containing proteins in liverwort morphogenesis. J Hattori Bot Lab 53: 221–227
4. Boundy JA, JS Wall, JE Turner, JH Woychik, RJ Dimler 1967 A mucopolysaccharide containing hydroxyproline from corn pericarp. J Biol Chem 242: 2410–2415
5. Chrispeels MJ 1969 Synthesis and secretion of hydroxyproline-containing macromolecules in carrots. I. Kinetic analysis. Plant Physiol 44: 1187–1193
6. Clark AE, RL Anderson, BA Stone 1979 Form and function of arabinogalactans and arabinogalactan-proteins. Phytochemistry 18: 521–540
7. Cleland R, AM Karlsnes 1967 A possible role of hydroxyproline-containing proteins in the cessation of cell elongation. Plant Physiol 42: 669–671
8. Engvall E, P Perlmann 1972 Enzyme-linked immunosorbant assay, ELISA. III. Quantitation of specific antibodies by enzyme-labeled anti-immunoglobulin in antigen-coated tubes. J Immunol 109: 129–135
9. Esquerre-Tugaye MT, D Mazau 1974 Effect of a fungal disease on extensin, the plant cell wall glycoprotein. J Exp Bot 25: 509–513
10. Jermyn MA, YM Yeow 1975 A class of lectins present in the tissues of seed plants. Aust J Plant Physiol 2: 501–531
11. Kieliszewski M, DTA Lamport 1986 Cross-reactivities of polyclonal antibodies against extensin precursors determined via ELISA techniques. Phytochemistry 25: 673–677
12. Kivirikko KI, M Liesmaa 1959 A colorimetric method for determination of hydroxyproline in tissue hydrolysates. Scand J Clin Lab Invest 11: 128–133
13. Laemmli UK, M Favre 1973 Maturation of the head of bacteriophage T4. J Mol Biol 80: 575–599
14. Lamport DTA 1965 The protein component of primary cell walls. Adv Bot Res 2: 151–218
15. Lamport DTA 1986 The primary cell wall: a new model. In RA Young, RM Rowell, eds, Cellulose: Structure, Modification, and Hydrolysis. John Wiley & Sons, New York, pp 77–90
16. Lamport DTA, DH Miller 1971 Hydroxyproline arabinosides in the plant kingdom. Plant Physiol 48: 454–456
17. Leach JE, MA Cantrell, L Sequeira 1982 Hydroxyproline-rich bacterial agglutinin from potato. Plant Physiol 70: 1353–1358
18. McIlvaine 1921 A buffer solution for colorimetric comparison. J Biol Chem 49: 183–186
19. Mellon JE, JP Helgeson 1982 Interaction of a hydroxyproline-rich glycoprotein from tobacco callus with potential pathogens. Plant Physiol 70: 401–405
20. Murashige T, F Skoog 1962 A revised medium for rapid growth and bioassay with tobacco tissue culture. Physiol Plant 15: 473–497
21. Roberts K, C Grief, GJ Hills, PJ Shaw 1985 Cell wall glycoproteins structure and function. J Cell Sci Suppl 2: 105–127
22. Showalter AM, JE Varner 1986 Biology and molecular biology of plant hydroxyproline-rich glycoproteins. *In* A Marcus, ed, The Biochemistry of Plants: A Comprehensive Treatise, Vol 11: Molecular Biology. Academic Press, New York
23. Showalter AM, JN Bell, CL Cramer, JA Bailey, JE Varner, CJ Lamb 1985 Accumulation of hydroxyproline-rich glycoprotein mRNAs in response to fungal elicitor and infection. Proc Natl Acad Sci USA 82: 6551–6555
24. Smith JJ, EP Muldoon, DTA Lamport 1984 Isolation of extensin precursors by direct elution of intact tomato cell suspension cultures. Phytochemistry 23: 1233–1239
25. Smith JJ, EP Muldoon, JJ Willard, DTA Lamport 1986 Tomato extensin precursors P1 and P2 are highly periodic structures. Phytochemistry 25: 1021–1030
26. Stafstrom J, LA Staehelin 1986 Cross-linking patterns in salt-extractable extensin from carrot cell walls. Plant Physiol 81: 234–241
27. Tyler JM, D Branton 1980 Rotary shadowing of extended molecules dried from glycerol. J Ultrastruct Res 71: 95–102
28. Van Etten CH, RW Miller, IA Wolff, Q Jones 1963 Amino acid composition of seeds from 200 angiospermous plant species. Agric Food Chem 11: 399–410
29. Van Holst G, JE Varner 1984 Reinforced polyproline II conformation in a hydroxyproline rich cell wall glycoprotein from carrot root. Plant Physiol 74: 247–251
30. Wray W, T Boulikas, VP Wray, R Hancock 1981 Silver staining of protein in polyacrylamide gels. Anal Biochem 118: 197–203