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Extracellular Polysaccharides and Proteins of Tobacco Cell Cultures and Changes in Composition Associated with Growth-Limiting Adaptation to Water and Saline Stress[1]

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ABSTRACT

The chemical composition of extracellular polymers released by cells of tobacco (*Nicotiana tabacum* L. cv W38) adapted to a medium containing 30% polyethylene glycol 8000 (−28 bar) or 428 millimolar NaCl (−23 bar) was compared to the composition of those released by unadapted cells. Unadapted cells released uronic acid-rich material of high molecular weight, arabinogalactan-proteins, low molecular weight fragments of hemicellulosic polysaccharides, and a small amount of protein. Cells adapted to grow in medium containing NaCl released arabinogalactan and large amounts of protein but not the uronic acid-rich material, and cells adapted to grow in polyethylene glycol released only small amounts of an arabinogalactan of much lower molecular weight and some protein. Secretion of all material was nearly blocked by polyethylene glycol, but when cells were transferred to a medium containing iso-osmolar mannitol, they again released extracellular polymers at rates similar to those of unadapted cells. Like cells adapted to NaCl, however, these cells released arabinogalactan and large amounts of protein but only small amounts of the uronic acid-rich material. Media of NaCl-adapted cells were enriched in 40, 29, and 11 kilodalton polypeptides. $CaCl_2$ extracted the 40 and 11 kilodalton polypeptides from walls of unadapted cells, but the 29 kilodalton polypeptide was found only in the medium of the NaCl-adapted cells. Accumulation of low molecular weight polysaccharide fragments in the medium was also substantially reduced in both NaCl- and polyethylene glycol-adapted cells, and specifically, the material was composed of lower proportions of xyloglucan fragments. Our results indicate that adaptation to saline or water stress results in inhibition of both the hydrolysis of hemicellulosic xyloglucan and release of uronic acid-rich material into the culture medium.

Cells in suspension culture adapted to grow in media mimicking saline or water stress partition more carbon into osmotic pools at the expense of the synthesis of cell wall (17). Cells adapted to either 30% PEG or 428 mM NaCl accumulate dry weight at rates comparable to unadapted cells but exhibit as much as a 5-fold decrease in average cell volume (3, 4) and a reduced rate of cell expansion (20). This occurs despite an osmotic "overadjustment" that results in development of turgor pressures severalfold higher than in unadapted cells (3, 4, 17). These observations suggest that changes in extensibility or other mechanical properties of the cell wall are responsible for this self-imposed failure of adapted cells to expand. In companion studies, we found that loss of tensile strength was correlated with a reduction in the mass of the cellulose-extensin network (20) and that substantial alterations in the chemical composition and organization of the pectic substances in walls of adapted cells indirectly may participate in reducing the ability of the cell wall to extend (21). Cell suspension cultures also secrete or release polysaccharides and protein that may reach 20% of the dry weight of the cells (13). These ECPs[3] are composed of mainly AGP, uronic acid-rich material, and smaller amounts of protein and small polysaccharides, and oligosaccharide fragments with chemical composition resembling hemicellulosic polysaccharides found in the primary cell wall (1, 2, 13, 19). Many models predict that enzymes localized in the cell wall participate in hydrolysis of the glycosidic load-bearing bonds of matrix polysaccharides and are the determinants of wall loosening and expansion (9, 22, 32, 33, 35). During the expansion of cells in suspension culture, the products of such hydrolyses are likely to escape into the medium (13, 14, 30, 33).

We examined the influence of adaptation to NaCl- and PEG-induced stress on the rates of secretion of ECPs and their chemical composition to document the relationship between the release of ECPs and cell growth and turnover of structural polymers in the cell walls of adapted and unadapted cells.

MATERIALS AND METHODS

Plant material

Cells of tobacco (*Nicotiana tabacum* L. cv W38) cells were maintained in liquid culture in MS medium (25) as described in the accompanying paper (20). Cells were adapted to grow in medium supplemented with either 30% PEG (−28 bar) or 428 mM NaCl (−23 bar) as described for tobacco and tomato (4, 6, 7) and have been maintained for several years prior to

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[3] Abbreviations: ECP, extracellular polymer; AGP, arabinogalactan-protein; HM,P, high molecular weight uronic acid-rich material.

use in experiments described here. In some experiments, cells in medium containing PEG were filtered aseptically, washed and resuspended in fresh medium for 15 min, refiltered, and resuspended again in medium containing iso-osmolar mannitol (0.86 M), and culture was continued for up to 4 d. Cell viability was determined intermittently with Evan's blue (15).

Isolation of ECP

At designated times during the culture cycle, medium was filtered through coarse sintered-glass funnels to remove cells and some debris. The filtered medium was brought to 80% (v/v) ethanol and chilled overnight at −20° to precipitate the extracellular material. Precipitates were washed with ice cold 80% ethanol, suspended in water, dialyzed overnight against running deionized water at ambient temperature, and lyophilized.

Gel Chromatography

Samples of lyophilized ECP (about 20 mg) were dissolved by stirring and sonication in a warm water bath in 10 mL of 0.2 M Tris-HCl (pH 8.6) supplemented with 0.2 M KCl, and small amounts of undissolved material were removed by centrifugation. This supernatant was loaded onto a 2.5-cm × 60-cm column of Sepharose 4B-200 (Pharmacia, repackaged by Sigma) equilibrated in the same buffer, and 5-mL fractions were collected. Estimation of the molecular mass of the ECP fractions was made with branched dextrans 17.5 to 500 kD (Sigma). Two major extracellular fractions containing neutral sugar from unadapted and NaCl-adapted cells and the single neutral sugar fraction from PEG-adapted cells were dialyzed extensively against running deionized water, lyophilized, and analyzed separately. Transfer of PEG-adapted cells to iso-osmotic mannitol resulted in release of material of higher weight containing neutral sugar, and two fractions based on size comparable to those from unadapted and NaCl-adapted cells were collected.

Chemical Analysis of ECP and Determination of Sugar Composition and Linkage Analysis

Protein, total sugar, and uronic acids in samples of crude and fractionated material were assayed as described in the accompanying paper (20). For neutral sugar composition, polymers were hydrolyzed in 1 mL of 2 M TFA containing 1 μmol *myo*-inositol (internal standard) at 120°C for 90 min. Reduction and acetylation of the sugars and the separation of the derivatives were performed as described (21). Values reported are the means of samples from triplicate, but sometimes duplicate, experimental samples, and variance or standard deviation always less than ±5%.

For linkage analyses the carboxyl groups of the glycosyluronic acid were reduced by $NaBD_4$ after activation with carbodiimide according to Taylor and Conrad (34). Methylation of the polysaccharides, hydrolysis of the per-*O*-methylated polymers, and reduction, acetylation, and separation of their partially methylated derivatives were conducted as described previously (21). Values reported are the means of two samples, and variance was always less than ±5%.

Preparation of Ionically Bound Cell Wall Proteins and Medium Proteins for SDS-PAGE

Cell walls were prepared as described in the companion paper (20) with the omission of the SDS wash. Ionically bound cell wall proteins were eluted from the walls with 200 mM $CaCl_2$ (100 mL/100 g fresh weight of cells) by stirring for 3 h followed by filtration through nylon mesh. The volume of $CaCl_2$ eluate was reduced by partial lyophilization and then dialyzed against several changes of water for 24 h. The dialysate was lyophilized and redissolved in boiling SDS sample buffer (29).

The cell-free medium was collected by filtration of the cell culture through nylon mesh and lyophilized. The powder was dissolved in water and dialyzed against several changes of water for 24 h and lyophilized. This material was dissolved in boiling SDS sample buffer as described (29).

RESULTS

Rates of ECP Secretion

The release of ECP by both unadapted and NaCl-adapted cells was much greater than that by PEG-adapted cells (Fig. 1). The rate of release reached a maximum during exponential phase of growth. Release of all material was nearly blocked in PEG; when PEG-adapted cells were transferred after 23 d of culture into medium containing iso-osmotic mannitol, amounts of ECP increased about 10-fold in only 6 h (Fig. 2). The rate then dropped from 1.6 mg/h, observed during the first 6 h, to 0.1 mg/h thereafter. This rapid release of ECP indicated that much material had accumulated in the cell wall matrix, periplasmic space, or precipitated onto the surface of the wall and was rendered soluble, and subsequent slower

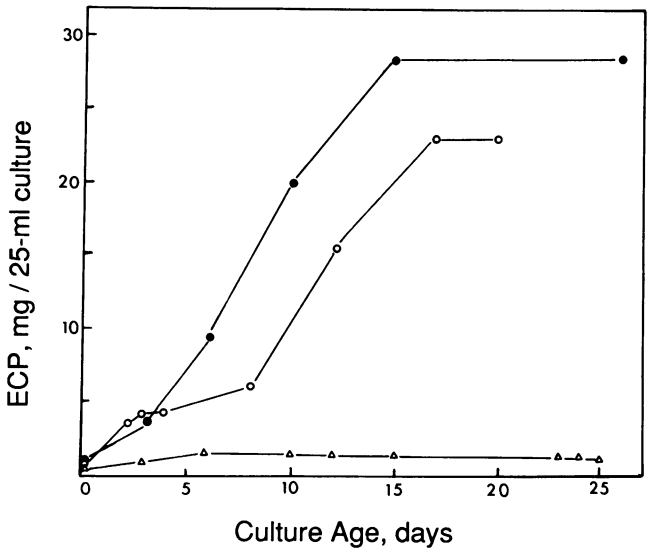

Figure 1. Release of extracellular polymers during the culture cycle of unadapted cells (○), cells adapted to 428 mM NaCl (●), and cells adapted to 30% PEG (△).

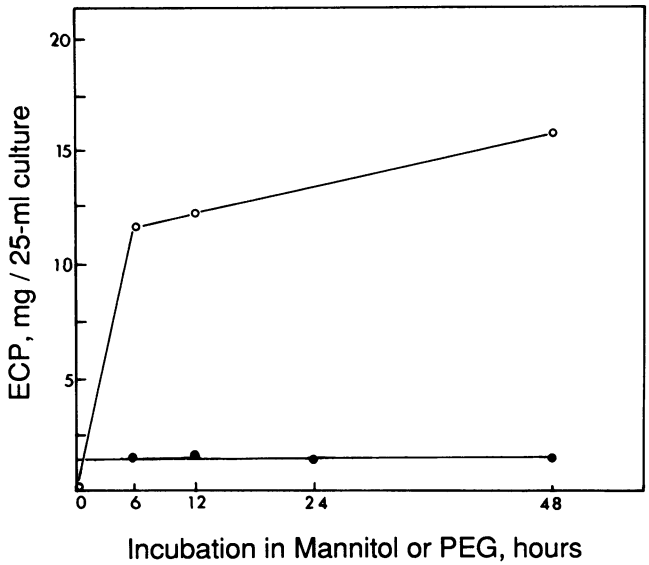

Figure 2. Release of extracellular polymers by PEG-adapted cells in PEG (●) and after transfer to medium containing iso-osmolar mannitol (○).

rates of release resulted from continued synthesis and extrusion (Fig. 2). Addition of PEG to medium from unadapted cells induced precipitation of much of the material (data not shown). Hence, blockage of secretion is likely a pharmacological effect of the PEG and not a physiological response to the stress. The composition of the materials from both PEG-adapted cells and from PEG-adapted cells transferred to iso-osmolar mannitol was compared to those from unadapted and NaCl-adapted cells.

Chemical Composition of ECP

The chemical composition of the ECP from both NaCl- and PEG-adapted cells differed from that of unadapted cells. The ECP from unadapted cells was enriched in uronic acid, whereas the ECP from NaCl-adapted cells was substantially enriched in protein (Table I). Transfer of PEG-adapted cells to medium containing mannitol resulted in marked release of neutral sugar and protein but only small amounts of uronic acid. The total ECP from unadapted cells contained predominantly arabinose, glucose, galactose, and xylose as neutral sugar constituents (Table II). Mainly arabinose and galactose and lower amounts of the other sugars comprised the neutral sugar from ECP from NaCl-adapted cells. The small amount of ECP from PEG-adapted cells was composed of mostly arabinose and galactose, and upon transfer to iso-osmotic mannitol, the proportion of arabinose increased as substantial amounts of material were released into the medium (Table II). Most notably, ECP from both lines of adapted cells contained markedly lower proportions of xylose and glucose (Table II).

Gel Chromatography

Because the ECPs were undoubtedly a mixture of many polymers, gel permeation chromatography of ECP on Sepharose 4B-200 was employed to reveal fundamental differences in the kinds of polymers released by adapted and unadapted cells. The ECP released by unadapted cells contained at least four major fractions of material: (a) a HM_rP that nearly voided the column; (b) a fairly homogeneous polysaccharide centered about 35 kD that contained both neutral sugars and uronic acids (fraction I); (c) a group of low mol wt polysaccharides and oligosaccharides (fraction II); and (d) protein eluting near the total included volume of the column (Fig. 3A). In contrast, the ECP from NaCl-adapted cells contained little HM_rP, but mostly the 35 kD polysaccharide (fraction I) and protein (Fig. 3B); the small amount of ECP from PEG-adapted cells was also devoid of HM_rP, and contained only protein and polysaccharides greatly reduced in size (Fig. 3C, fraction I). When the PEG-adapted cells were transferred to mannitol, the material released contained larger polysaccharides (fraction I) and large amounts of protein, but the cells released only small amounts of HM_rP (Fig. 4 A, B, and C). Very little HM_rP was released by transfer of PEG-adapted cells to mannitol indicating that this retention is not a result of mere precipitation by PEG.

Sugar and Linkage Analyses

In ECP from unadapted cells, the arabinose and galactose content of fraction I corresponding to the 35 kD polysaccharide (Fig. 3A) was about 70% of the total neutral sugar. Arabinose and galactose comprised 80% and 90% of the sugar in similar fractions of ECP from PEG- and NaCl-adapted cells, respectively; the proportion was unchanged upon transfer of the PEG-adapted cells to iso-osmotic mannitol (data not shown). Linkage analysis of fractions I from unadapted

Table I. *Amounts of Extracellular Material and Its Composition at Stationary Phase by Unadapted Cells, Cells Adapted to 428 mM NaCl, and Cells Adapted to 30% PEG before and after Transfer to Iso-osmotic Mannitol Medium*

Cell Type	Total ECP	Total Sugar	Uronic Acids	Protein
		mg/25 mL culture		
Unadapted	26.0	18.4	7.2	0.4
NaCl-adapted	28.0	24.1	1.1	2.8
PEG-adapted	1.0	0.8	0.1	0.1
PEG-adapted, transferred to mannitol for:				
6 h	11.8	8.2	0.9	2.7
12 h	12.2	7.8	1.1	3.3
48 h	15.8	10.4	1.8	3.6

Table II. *Neutral Sugar Composition of Total ECP Isolated from Cultures at Stationary Phase of Unadapted Cells, Cells Adapted to 428 mM NaCl, and Cells Adapted to 30% PEG before and after Transfer to Mannitol Medium for 12 h*

Source	Rhm	Ara	Xyl	Man	Gal	Glc
			mol %			
Unadapted cells	4	40	16	2	17	21
NaCl-adapted cells	3	60	4	3	22	8
PEG-adapted cells	5	32	4	10	39	10
PEG-adapted, transferred to mannitol medium	9	42	6	3	30	10

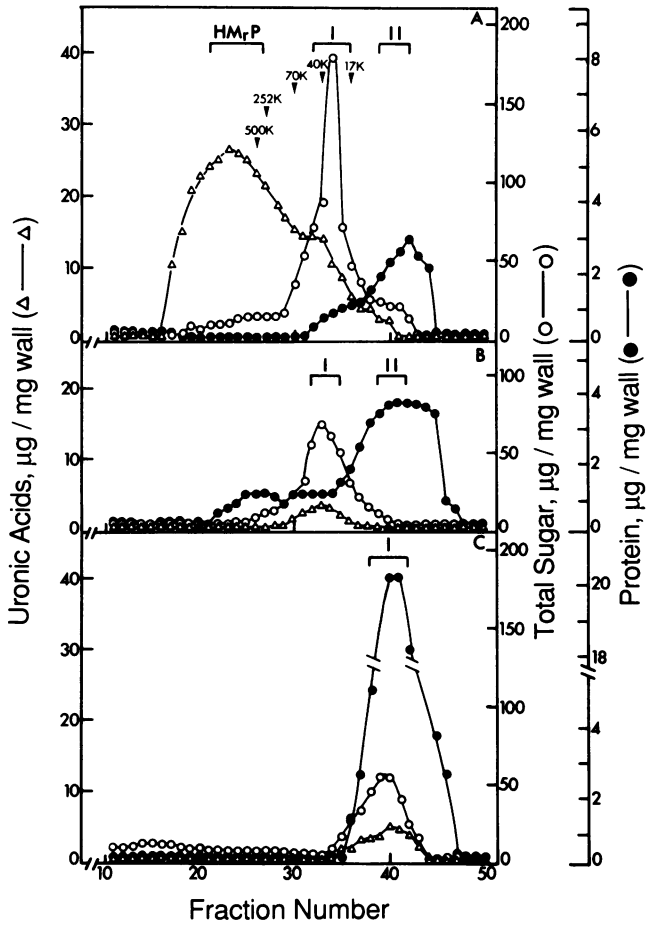

Figure 3. Gel chromatography on Sepharose 4B-200 of extracellular polymers from A, unadapted cells, B, cells adapted to 428 mM NaCl, and C, cells adapted to 30% PEG. Symbols are: (○) total sugar, (●) protein, and (△) uronic acid. Fractions I and II from unadapted cells represented that principal neutral sugar-containing material. Comparably sized fractions were taken from NaCl-adapted cells, but only a single fraction from PEG-adapted cells was taken. These fractions were dialyzed against deionized water and lyophilized, and linkage analyses of the two fractions from unadapted and NaCl-adapted cells are reported in Table III.

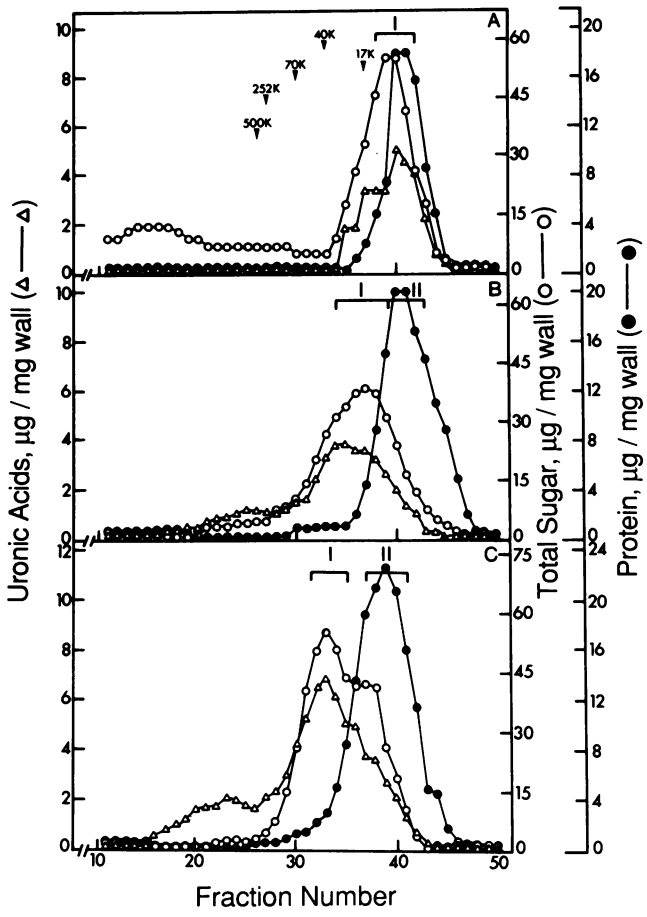

Figure 4. Gel chromatography on Sepharose 4B-200 of extracellular polymers from A, PEG-adapted cells, B, PEG-adapted cells transferred to mannitol medium for 12 h, and C, for 48 h. Symbols as in Figure 3. Fractions I and II were taken based on criteria in Figure 3, dialyzed and lyophilized, and linkage analyses on the two fractions from B are reported in Table III.

cells, NaCl-adapted cells (Fig. 3B), and PEG-adapted cells transferred to mannitol for 12 h (Fig. 4B) demonstrated that all materials contained predominantly non-reducing terminal, 2-, 3-, 5-, and 3,5-linked arabinofuranosyl and t-, 3-, 6-, and 3,6-linked galactopyranosyl units (Table III). These sugar linkages are expected from type II arabinogalactan isolated from cell suspension cultures of tobacco and many other species (13, 19). The unadapted cells also contained substantial amounts of low mol wt polysaccharides and oligosaccharides (Fig. 3A, fraction II). This fraction also contained linkages typical of arabinogalactans, but terminal- and 2-linked xylosyl and 4- and 4,6-linked glucosyl units typical of dicot xyloglucans (2) and 4-, 2,4-, and 3,4-linked xylosyl units of dicot arabinoxylans (12) were also in this fraction (Table III). The amounts of this material were substantially lower in either NaCl-adapted cells (Fig. 3B) or PEG-adapted cells transferred

Table III. *Linkage Analysis of Fractions I and II from Cultures at Stationary Phase of Unadapted, NaCl-Adapted (Fig. 3 A and B), and PEG-Adapted Cells Transferred to Medium Containing Iso-osmotic Mannitol for 12 h (Fig. 4 B)*

Sugar Linkage[a]	Fraction and Cell Line					
	I			II		
	Unadapted	NaCl-adapted	PEG-adapted	Unadapted	NaCl-adapted	PEG-adapted
	mol %					
t-Rhm*p*	1	tr[b]	tr	tr	tr	tr
2-Rhm*p*	5	2	1	tr	1	1
2,4-Rhm*p*	2				1	
	8	2	1	tr	2	1
t-Fuc		tr	1	tr	tr	1
t-Ara*f*	31	37	26	22	22	30
t-Ara*p*	2	tr	tr	1	1	1
2-Ara*f*	2	11	2	1	1	1
3-Ara*f*	2	7	4	1	1	
5-Ara*f*	12	16	14	5	9	11
2,5-Ara*f*	1					
3,5-Ara*f*	3	tr	1			
	53	71	47	30	34	43
t-Xyl*p*	2	1	4	11	7	6
2-Xyl*p*	2	1	3	5	2	2
2,3+3,4-Xyl*p*	2	1	1	tr	1	1
2,4-Xyl*p*	tr	1	1	1	1	1
4-Xyl*p*	3	tr	3	10	7	9
	9	4	12	27	18	19
t-Man*p*		tr	1	1	1	2
4-Man*p*			1	1	2	1
6-Man*p*		tr		1		
4,6-Man*p*			tr	1	3	1
			2	4	6	4
t-Glc*p*	3	tr	1	2	3	2
2-Glc*p*		tr	7	–	tr	1
4-Glc*p*	6	2	–	13	19	7
4,6-Glc*p*	6	1	4	10	6	3
	15	3	12	25	28	13
t-Gal*p*	3	1	5	2	3	4
2-Gal*p*			1	6		
3-Gal*p*		5	7	1	2	4
4-Gal*p*	2		tr		tr	
6-Gal*p*	1	2	3	1	1	3
3,6-Gal*p*	9	12	9	4	6	8
	15	20	25	14	12	19

[a] Linkages were deduced from electron-impact mass spectrometry of partially methylated alditol acetate derivatives. t-=nonreducing terminal sugar; 2*p*-=2-linked rhamnopyranosyl unit deduced from 1,2,5-tri-*O*-acetyl-(1-deuterio)-3,4-di-*O*-methyl-6-deoxyhexitol identified by Gc-ms (21), and so forth. [b] tr=trace amounts less than 0.5%.

to mannitol (Fig. 4, A, B, and C). We had observed that the total extracellular material from NaCl- and PEG-adapted cells had greatly reduced proportions of polysaccharides containing xylose and glucose compared to that from unadapted cells (Table II). Unadapted cells released xyloglucan fragments (Fig. 3A, fraction II; Table III) and NaCl-adapted cells released very little (Fig. 3B, fraction II; Table III). PEG-adapted cells transferred to mannitol released polysaccharide of a mol wt similar to that of the xyloglucan-enriched fractions (Fig. 4C), but these polysaccharides, based on linkage analysis, contained much less xyloglucan (Table III).

SDS-PAGE of Ionically Bound Wall Proteins and Proteins of ECP

The saline-adapted cells and the PEG-adapted cells transferred to iso-osmolar mannitol released 7- and 9-fold more protein into the incubation medium than unadapted cells (Table I). Amino acid analyses revealed few qualitative differences in the total amino acid composition of the released proteins from adapted and unadapted cells. In NaCl-adapted cells, the amounts of hydroxyproline in protein found in the medium varied but was substantial, accounting for about 26%

of the total amino acids (Table IV). Despite similar proportions of the amino acids, SDS-PAGE revealed marked differences in the distribution of $CaCl_2$-extractable proteins from the wall and those released to the extracellular medium (Fig. 5). The $CaCl_2$-extract of cell walls purified from unadapted cells at mid-logarithmic growth was predominated by 40, 23, and 11 kD polypeptides. Lesser amounts of 51, 20, and 17 kD proteins were also observed. The saline-adapted cells, however, had substantially reduced proportions of the 40 kD polypeptide, and the 51 and 17 kD polypeptides were not detected. The salt-adapted cells also had substantially increased amounts of the 20 kD and the 11 kD polypeptides. By comparison, the incubation medium of the salt-adapted cells contained a unique 29 kD protein not found in either the cell walls or medium from unadapted cells. Substantial amounts of apparent 40 kD and 11 kD protein were found in the medium of the salt-adapted cells—an indication that many of these proteins had been displaced from the cell wall. Some of the 40 kD and 11 kD proteins were also observed in medium from unadapted cells, but the 23 kD polypeptide found in cell walls of both unadapted and salt-adapted cells was held tenaciously and very little was released to the medium (Fig. 5).

DISCUSSION

The inhibition of ECP secretion in cells adapted to PEG is probably a pharmacological effect of PEG rather than a result of decreased water potential of the medium or a specific metabolic inhibition. Polyethylene glycol does not penetrate the cell wall (8), and despite some claims for toxic effects of its contaminants (16, 23), the purified PEG available commercially has been used without physiological impairments in various plant species such as tomato cell cultures (6, 7, 17), excised pine xylem tissue (37), and detached soybean ovules (27). Transfer of cells from PEG into medium containing mannitol had no immediate effect on cell viability; viability did not drop below 80% during 4 d of incubation (data not shown). Mannitol is metabolized by certain plant tissues (36), but comparable rates of mannitol uptake and metabolism in tobacco cells would be too slow to have caused a substantial change in the osmotic potential of the medium during the short incubations.

Table IV. *Amino Acid Composition (as mol % of Total Amino Acids) of Lyophilized Material from the Incubation Medium of NaCl-Adapted and Unadapted cells*

Medium was taken at stationary phase of culture.

Amino Acid	Cell Type		Amino Acid	Cell Type	
	S0	S25		S0	S25
Alanine	11.6	13.4	Methionine	tr[a]	tr
Glycine	11.3	9.4	Asn + Asp	6.7	5.3
Valine	5.1	6.5	Phenylalanine	2.8	2.8
Threonine	5.2	3.1	Gln + Glu	6.2	4.5
Serine	7.0	2.6	Lysine	6.9	9.1
Leucine	5.8	4.6	Tyrosine	0.8	tr
Isoleucine	1.9	2.7	Arginine	2.4	2.0
Proline	8.7	7.9	Histidine	tr	0.2
Hydroxyproline	16.9	26.1	Cystine	tr	tr

[a] tr = trace amounts less than 0.1%.

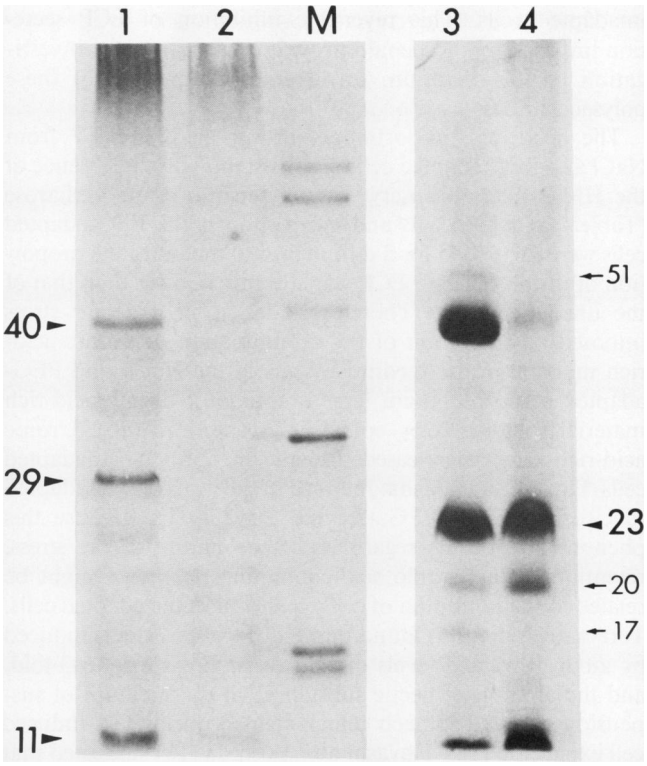

Figure 5. SDS-PAGE of tobacco proteins. Lanes 1 and 2 are proteins released into the medium by cells adapted to 428 mM NaCl and unadapted cells, respectively. Each lane contains an extract of equal amounts of lyophilized extracellular material taken from cultures at mid-logarithmic growth phase. Markers are: ovotransferrin, 77 kD; bovine serum albumin, 66.3 kD, ovalbumin, 45 kD; carbonic anhydrase, 30 kD; myoglobin, 17.2 kD; and cytochrome c, 12.3 kD. Lanes 3 and 4 are proteins extracted with 200 mM $CaCl_2$ from purified cell walls of unadapted and adapted cells, respectively. Each lane represents an extract containing equal amounts of protein.

The inhibition of the secretion of ECP in PEG-medium and its renewal upon transfer of the cells to medium containing mannitol (Figs. 1 and 2) suggested that the secretion of ECP was blocked by PEG *per se* and not by low water potential of the medium. Precipitation of ECP onto the surface of the cell wall could simply decrease porosity of the wall and prevent further secretion of polymers, particularly those of higher mol wt, and these polymers may have accumulated in the wall and periplasmic space. We found that PEG precipitated ECP from medium of unadapted cells (data not shown). Coté and colleagues (11) also observed that PEG decreases diffusion of larch AGP from sectioned material, and Paull and Jones (26) have suggested that addition of polygalacturonic acid and other charged polymers to cultured sycamore cells block the secretion of ECP by decreasing wall porosity. The small amount of AGP secreted into the PEG medium was of lower mol wt (Fig. 3 c) and is consistent with restricted wall porosity. Removal of the block to secretion by transferring the cells into iso-osmolar mannitol medium resulted in either release of the ECP, including some HM_rP from the cell wall (Fig. 4, B and C, and table II), or renewed synthesis of the polysaccharide (Fig. 2). Further, the released polymer is of larger molecular size and similar to that of the

unadapted cells. This reversible inhibition of ECP secretion imposed by PEG could provide a useful tool for investigation of the secretion, turnover, and function of these polysaccharides.

The decreased proportion of uronic acids in ECP from NaCl and PEG-adapted cells is consistent with the absence of the HM_rP fraction observed upon separation on Sepharose (Table I and Fig. 3, B and C). Although the PEG-adapted cells were incubated for 2 d in mannitol medium, the proportion of uronic acids in ECP was still much lower than that of the unadapted cells. Therefore, adaptation to water stress imposed an inhibition of the accumulation of uronic acid-rich material in the medium. Walls of the NaCl- and PEG-adapted cells do contain large amounts of uronic acid-rich material solubilized by cold EDTA solutions (20). Uronic acid-rich material released into the medium by unadapted cells (Fig. 3A) may be that retained in the walls of cells adapted to either NaCl or PEG (see ref. 20, Fig. 1). Because this phenomenon occurs regardless of the nature of the stress, retention of the uronic acid-containing polymers might be related to the inhibition of cell expansion in the adapted cells. Terry and Jones (35) found that growth of pea stems induced by auxin increased levels of soluble pectins by 2- to 3-fold, and the amount of pectic substances in the medium of suspension-cultured spinach cells increased during GA-induced cell expansion (14). Hayashi and Yoshida (18) suggested that fragments of uronic acid-rich polymers may have additional growth regulating properties.

In all cell lines, arabinose and galactose were predominant sugars, an indication of arabinogalactan (Table II). Analysis of the linkages present in fraction I from Sepharose 4B-200 chromatography (Figs. 3, A and B, and 4C) confirmed the presence of this polymer since the terminal and 5-linked arabinofuranosyl and the 3,6-linked galactopyranosyl residues are predominant in this fraction from all cell lines (Table III). Although the release of this polymer was blocked by PEG, PEG-adapted cells transferred to medium containing mannitol, and the NaCl-adapted cells released about the same amount of total sugar, with almost equal proportions of AGP, as the unadapted cells (Table I and III). The secretion of AGP is typical of suspension cultures (1, 13, 19), but the function of this polymer is still obscure.

Bozarth et al. (5) reported the extraction of a 28 kD polypeptide from walls of water-stressed soybean seedlings. Our preliminary analyses of the amino acid composition of the 29 kD extracellular protein demonstrated some similarities, but we await further characterization of both of these polypeptides to evaluate their homology and possible function. Based on studies of cross-reactivity with antibodies (data not shown), the 29 kD extracellular protein is not osmotin, a thaumatin-like protein associated with adaptation to NaCl (28, 29). While increase in the amount of these extracellular and wall proteins is marked in the adapted cells, none of these separated by SDS-PAGE is greatly enriched in Hyp (data not shown). Considering that Hyp constitutes almost 20% of the total wall and extracellular amino acids (Table IV), much of the material could be soluble extensin precursors that fail to polymerize. These precursors are cationic even in SDS solution because of their polyproline II-like structure (10, 24) and must be characterized by cationic-urea gel systems or the equivalent (31). A less likely possibility is that the peptide moiety of AGP, which is also a hydroxyproline-rich protein, is increased markedly in abundance in the adapted cells. Equal amounts of the AG carbohydrate accumulate in both unadapted and adapted cells, however, and the large amounts of protein found in the adapted cells are resolved from the AGP (Figs. 3, A and B, and 4C). These adapted cells are potentially useful for generating large amounts of precursors of wall structural proteins for study. Since neither the identity nor the origin of the proteins in ECP is known, the physiological nature of the increased secretion of these proteins by the adapted cells (Table I) is yet to be explained. Purification and characterization of these extracellular proteins are perhaps prerequisites for understanding their functions and their relation to adaptation to osmotic stress.

From analysis of sugars in the total extracellular material, we observed that adapted cells had greatly reduced proportions of xylose- and glucose-containing polymers (Table II). Fraction II from the unadapted cells contained a small amount of carbohydrate (Fig. 3A), which was essentially absent from both NaCl-adapted and PEG-adapted cells (Figs. 3B and 4B), and much of this fraction contained linkages typical of xyloglucan (Table III). Our observation that unadapted cells contained substantial amounts of these putative fragments whereas the NaCl- and PEG-adapted did not indicated that the release of this polymer was substantially reduced upon adaptation to osmotic stress. Solubilization of polyuronides and xyloglucan was observed under conditions promoting cell expansion, such as hormone treatment (14, 35). Also, synthesis and degradation of hemicellulosic material were observed during the exponential phase of growth of *Vinca rosea* suspension culture (33). We suggest that the reduced amounts of soluble pectins and xyloglucan in the medium of both NaCl and PEG-adapted cells result from impaired turnover of matrix polysaccharides, and this impairment may account directly for the inhibition of growth resulting from adaptation to osmotic stress.

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LITERATURE CITED

1. **Aspinall GO, Molloy JA, Craig JWT** (1969) Extracellular polysaccharides from suspension-cultured sycamore cells. Can J Biochem **47:** 1063–1070
2. **Bacic A, Harris PJ, Stone BA** (1988) Structure and function of plant cell walls. *In* J Preiss, ed, The Biochemistry of Plants, Vol 14. Academic Press, New York, pp 297–371
3. **Binzel ML, Hasegawa PM, Handa AK, Bressan RA** (1985) Adaptation of tobacco cells to NaCl. Plant Physiol **79:** 118–125
4. **Binzel ML, Hasegawa PM, Rhodes D, Handa S, Handa AK, Bressan RA** (1987) Solute accumulation in tobacco cells adapted to NaCl. Plant Physiol **84:** 1408–1415
5. **Bozarth CS, Mullet JE, Boyer JS** (1987) Cell wall proteins at low water potentials. Plant Physiol **85:** 261–267

6. **Bressan RA, Handa AK, Handa S, Hasegawa PM** (1982) Growth and water relations of cultured tomato cells after adjustment to low external water potentials. Plant Physiol **70:** 1303–1309
7. **Bressan RA, Hasegawa PM, Handa AK** (1981) Resistance of cultured higher plant cells to polyethylene glycol-induced water stress. Plant Sci Lett **21:** 23–30
8. **Carpita NC, Sabularse D, Montezinos D, Delmer DP** (1979) Determination of the pore size of cell walls of living plant cells. Science **205:** 1144–1147
9. **Cleland RE** (1981) Wall extensibility: hormones and wall extension. *In* W Tanner, FA Loewus, eds, Encyclopedia of Plant Physiology (New Series), Vol 13B. Springer-Verlag, Berlin, pp 255–273
10. **Cooper JB, Chen JA, Van Holst G-J, Varner JE** (1987) Hydroxyproline-rich glycoproteins of plant cell walls. Trends Biochem Sci **12:** 24–27
11. **Coté WA Jr, Day AC, Simon BW, Timell TAE** (1966) Studies on larch arabinogalactan. I. The distribution of arabinogalactan in larch wood. Holzforschung **20:** 178–192
12. **Darvill JE, McNeil M, Darvill AG, Albersheim P** (1980) Structure of plant cell walls. XI. Glucuronoarabinoxylan, a second hemicellulose in primary cell walls of suspension-cultured sycamore cells. Plant Physiol **66:** 1135–1139
13. **Fincher GB, Stone BA, Clarke AE** (1983) Arabinogalactan-proteins: structure, biosynthesis, and function. Annu Rev Plant Physiol **34:** 47–70
14. **Fry SC** (1980) Gibberellin-controlled pectinic acid and protein secretion in growing cells. Phytochemistry **19:** 735–740
15. **Gaff DE, Okon'o-Ogloa O** (1971) The use of non-permeating pigments for testing the survival of cells. J Exp Bot **22:** 756–758
16. **Greenway H, Hiller RG, Flowers TJ** (1968) Respiratory inhibition in *Chlorella* produced by "purified" polyethylene glycol 1540. Science **159:** 984–985
17. **Handa S, Bressan RA, Handa AK, Carpita NC, Hasegawa PM** (1983) Solutes contributing to osmotic adjustment in cultured plant cells adapted to water stress. Plant Physiol **73:** 834–843
18. **Hayashi T, Yoshida K** (1988) Cell expansion and single-cell separation induced by colchicine in suspension-cultured soybean cells. Proc Natl Acad Sci USA **85:** 2618–2622
19. **Hori H, Takeuchi Y, Fujui T** (1980) Structure of an arabinogalactan of extracellular hydroxyproline-rich glycoprotein in suspension-cultured tobacco cells. Phytochemistry **19:** 2755–2756
20. **Iraki NM, Bressan RA, Hasegawa PM, Carpita NC** (1989) Alteration of the physical and chemical structure of the primary cell wall of growth-limited plant cells adapted to osmotic stress. Plant Physiol **91:** 39–47
21. **Iraki NM, Singh N, Bressan RA, Carpita NC** (1989) Cell walls of tobacco cells and changes in composition associated with reduced growth upon adaptation to water and saline stress. Plant Physiol **91:** 48–53
22. **Labavitch JM, Ray PM** (1974) Relationship between promotion of xyloglucan metabolism and induction of elongation by IAA. Plant Physiol **54:** 499–502
23. **Lagerwerff JW, Ogata G, Eagle HE** (1961) Control of osmotic pressure of culture solution with polyethylene glycol. Science **133:** 1486–1487
24. **Lamport DTA, Epstein L** (1983) A new model for the primary cell wall: A concatenated extensin-cellulose network. *In* DD Randall, DG Blevins, RL Larson, BJ Rapp, eds, Current Topics in Plant Biochemistry and Physiology, Vol 2. University of Missouri, Columbia, pp 73–83
25. **Murashige T, Skoog F** (1962) A revised medium for rapid growth and bioassay with tobacco tissue culture. Physiol Plant **15:** 473–497
26. **Paull RE, Jones RL** (1978) Regulation of synthesis and secretion of fucose-containing polysaccharides in cultured sycamore cells. Aust J Plant Physiol **5:** 457–467
27. **Picaard CS, Cherry JH** (1984) Maintenance of normal supranormal protein accumulation in developing ovules of *Glycine max* L. Merr. during PEG-induced water stress. Plant Physiol **75:** 176–180
28. **Singh NK, Bracker CA, Hasegawa PM, Handa AK, Buckel S, Hermodsen HA, Pfankoch E, Regnier FE, Bressan RA** (1987) Characterization of osmotin, a thaumatin-like protein associated with osmotic adaptation in plant cells. Plant Physiol **85:** 529–536
29. **Singh NK, LaRosa PC, Handa AK, Hasegawa PM, Bressan RA** (1987) Hormonal regulation of protein synthesis associated with salt tolerance in plant cells. Proc Natl Acad Sci USA **84:** 739–743
30. **Stevenson TT, McNeil M, Darvill AG, Albersheim P** (1986) Structure of plant cell walls. XVIII. An analysis of the extracellular polysaccharides of suspension cultured sycamore cells. Plant Physiol **80:** 1012–1019
31. **Stuart DM, Varner JE** (1980) Purification and characterization of a salt-extractable hydroxyproline-rich glycoprotein from aerated carrot discs. Plant Physiol **66:** 787–792
32. **Taiz L** (1984) Plant cell expansion: regulation of cell wall mechanical properties. Annu Rev Plant Physiol **35:** 585–657
33. **Takeuchi Y, Komamine A** (1980) Turnover of cell wall polysaccharides of a *Vinca rosea* suspension culture. I. Synthesis and degradation of cell wall components. Physiol Plant **48:** 271–277
34. **Taylor RL, Conrad HE** (1972) Stoichiometric depolymerization of polyuronides and glycosaminoglycuronans to monosaccharides following reduction of their carbodiimide-activated carboxyl groups. Biochemistry **11:** 1383–1388
35. **Terry ME, Jones RL** (1981) Soluble cell wall polysaccharides released from pea stems by centrifugation. I. Effect of auxin. Plant Physiol **68:** 531–537
36. **Thompson MR, Douglas TJ, Obata-Sasamoto H, Thorpe TA** (1986) Mannitol metabolism in cultured plant cells. Physiol Plant **67:** 365–369
37. **Whitmore FW, Zahne R** (1967) Evidence for a direct effect of water stress on tracheid cell wall metabolism in pine. For Sci **13:** 397–400