Plant Physiol. (1993) 103: 987-992

Xyloglucan Endotransglycosylase Activity in Carrot Cell Suspensions during Cell Elongation and Somatic Embryogenesis[1]

P. Richard Hetherington and Stephen C. Fry*

Centre for Plant Science, Division of Biological Sciences, University of Edinburgh, Daniel Rutherford Building, The King's Buildings, Mayfield Road, Edinburgh, EH9 3JH, United Kingdom

Xyloglucan endotransglycosylase (XET) has been proposed to contribute to cell elongation through wall loosening. To explore this relationship further, we assayed this enzyme activity in suspensions of carrot (*Daucus carota* L.) cells exhibiting various rates of cell elongation. In one cell line, elongation was induced by dilution into dichlorophenoxyacetic acid (2,4-D)-free medium. During this elongation, 93% of the XET activity was found in the culture medium; in nonelongating controls, by contrast, 68% was found in the cell extracts even though the specific activity of these extracts was lower than in the elongating cells. By far the highest rates of XET secretion per cell were in the elongating cells. A second cell line was induced to undergo somatic embryogenesis by dilution into 2,4-D-free medium. During the first 6 d, numerous globular embryoids composed of small, isodiametric cells were formed in the absence of cell elongation; extracellular XET activity was almost undetectable, and intracellular specific activity markedly declined. After 6 d, heart, torpedo, and cotyledonary embryoids began to appear (i.e. cell elongation resumed); the intracellular specific activity of XET rose rapidly and >80% of the XET activity accumulated in the medium. Thus, nonexpanding cell suspensions (whether or not they were rapidly dividing) produced and secreted less XET activity than did expanding cells. We propose that a XET molecule has an ephemeral wall-loosening role while it passes through the load-bearing layer of the wall on its way from the protoplast into the culture medium.

Wall extensibility is often the controlling factor in cell expansion (e.g. Cleland, 1977; Taiz, 1984). However, the underlying biochemistry involved in the control of extensibility, a physical property, is not fully understood.

Two major components of the dicotyledonous primary cell wall are cellulose microfibrils and xyloglucan, which are thought to form a network with the microfibrils being tethered by xyloglucan chains through hydrogen bonding (Fry, 1989; McCann et al., 1990). Moreover, in a turgid cell such chains could be load bearing and hence limiting to cell expansion. Support for this idea comes from studies demonstrating that, during growth, alterations to xyloglucan occur that could contribute to an increase in wall extensibility. Examples include an auxin- or acid-promoted release of xyloglucan fragments from the wall (Labavitch and Ray, 1974; Gilkes and Hall, 1977) and decreased mol wt of the remaining polymeric xyloglucan (Hayashi and Maclachlan, 1984; Lorences and Zarra, 1987), partial dissolution of wall-bound xyloglucan (Terry et al., 1981), and the inhibition of auxin-induced elongation by both lectins (Hoson and Masuda, 1987) and antibodies (Hoson et al., 1991) that bind to xyloglucan.

Within the xyloglucan-cellulose network, wall loosening could be brought about by cleavage of xyloglucan tethers by cellulase and/or XET. Cellulase (β-[1→4]-D-glucanase) catalyzes the irreversible cleavage of xyloglucan chains by endohydrolysis (Hayashi et al., 1984), but the strength contributed by the tether would be lost. XET activity has been found in extracts from the growing portions of monocots and dicots and also in cell suspensions (Baydoun and Fry, 1989; Smith and Fry, 1991; Farkaš et al., 1992; Fry et al., 1992b; Nishitani and Tominaga, 1992). This enzyme cleaves a xyloglucan chain and could therefore allow wall expansion but, unlike cellulase, XET transfers the cut (potentially reducing) end onto the nonreducing terminus of a chemically similar xyloglucan molecule. A new tether could thus be formed, restoring much of the wall's original strength. A 33-kD protein with this activity and lacking hydrolase activity has been purified from the apoplastic fluid of *Vigna* (Nishitani and Tominaga, 1992). A different enzyme has been purified from nasturtium cotyledons and found to be able to catalyze both the hydrolysis of xyloglucan and, at high concentrations of suitable acceptor substrates, its transglycosylation (Fanutti et al., 1993).

In this study we have examined whether XET is associated with cell expansion by assaying this enzyme in two different carrot (*Daucus carota* L.) cell suspension systems that can be manipulated to change cell size and shape. In one instance a high-density, nonembryogenic suspension of small, isodiametric cells (the Oxford cell line) was diluted into auxin-free medium, resulting in substantial cell elongation. In the other system, a similar treatment applied to PEMs (clusters of 10–20 tightly adhering, densely cytoplasmic cells) obtained from an embryogenic suspension (line 10) led to the formation of somatic embryoids. During embryogenesis there is an initial requirement for cells of the PEMs to remain small and tightly

[1] Financial support was provided by the European Community BRIDGE program.

* Corresponding author; fax 44-31-650-5392.

Abbreviations: PEM, proembryogenic mass; XET, xyloglucan endotransglycosylase; XG7, xyloglucan heptasaccharide ($Glc_4 \cdot Xyl_3$); XG7-ol, the oligoglycosyl alditol obtained by reduction of XG7.

adhering so as to initiate globular embryoids successfully (Cordewener et al., 1991). However, at the heart and torpedo stages of embryogenesis, localized cell elongation resumes.

MATERIALS AND METHODS

Cell Culture Systems

Cell Elongation

A nonembryogenic, high-density suspension of carrot cells (*Daucus carota* L.), the Oxford cell line (designated L2 in Knox et al., 1991), containing over 90% small, isodiametric cells was subcultured by 10-fold dilution every 7 d into Murashige and Skoog medium (Murashige and Skoog, 1962) with 0.5 mg/L 2,4-D and 25 g/L Suc. Elongation was induced by 100-fold dilution (of 1 mL of 5-d-old suspension) into the above medium without 2,4-D. A control culture was diluted 10-fold into medium containing 2,4-D. The suspensions were then incubated in darkness at 25°C on an orbital shaker at 60 rpm for 5 d. During this time, packed cell volume (after centrifugation at 1000*g* for 5 min) and cell number (using a hemocytometer) were measured.

Somatic Embryogenesis

A high-density suspension of an embryogenic line (line 10 of De Vries et al., 1988) was subcultured every 14 d by dilution of 2 mL of packed cell volume into 50 mL of B5 medium containing 0.44 mg/L 2,4-D (Gamborg et al., 1968). Somatic embryos were produced from a 7-d-old suspension by dilution of a fraction enriched in PEMs (50–125 μm aggregates, isolated by sieving) into 2,4-D-free medium according to De Vries et al. (1988). As a control, PEMs were diluted to the same extent but into 2,4-D-containing medium. The cell number immediately after dilution in both cases was about 20,000 cells/mL. The number and developmental stages of embryoids were recorded over 12 d.

Throughout all experiments cell viability was >95%, as observed by fluorescence microscopy after staining with fluorescein diacetate (Widholm, 1972).

Substrates

Xyloglucan was purified from *Tropaeolum majus* seeds by Cu^{2+} precipitation (Rao, 1959). XG7 was prepared and purified from *Rosa* cell suspensions (Lorences and Fry, 1993). [1-^{3}H]XG7-ol was produced by NaB^3H_4 reduction of XG7. The oligosaccharide (2 mg) was reacted with 925 MBq NaB^3H_4 (278 TBq/mol) in 250 μL of 1 M NH_3 at room temperature overnight. Unreacted borohydride was destroyed by addition of 250 μL of 5 M acetic acid; the reaction mixture was left, with occasional shaking, for 24 h in a fume cupboard for complete removal of $^3H_2$ gas. The reaction mixture was made to 1 mL with acetic acid:pyridine:H_2O (1:1:98, v/v) and the radioactive alditol product was collected after gel-permeation chromatography (on Bio-Gel P-2). The specific activity of [^{3}H]XG7-ol was 22 TBq/mol.

Extraction of Enzyme Activities

Both intracellular and extracellular activities of XET were assayed. For extracellular measurements, cells were removed from the medium by centrifugation (1000*g* for 5 min) and protein in the supernatant was concentrated by precipitation (at 0°C) with 65% saturated $(NH_4)_2SO_4$ after the addition of 2 mg/mL BSA (Sigma) as carrier [no additional activity was obtained using higher $(NH_4)_2SO_4$ concentrations]. After centrifugation at 25,000*g* for 30 min, the washed protein pellet was resuspended in 10 mM $CaCl_2$, 10 mM ascorbate, 1 mM DTT, and 50 mM phthalate (Na^+) buffer, pH 5.5, for XET assays. Desalting was unnecessary because enzyme activity was unaffected by $(NH_4)_2SO_4$ at the remaining concentrations.

For intracellular measurements, cells were recovered from the medium by centrifugation, washed, and homogenized by sonication for 2 × 1 min (MSE Soniprep, 4-mm diameter probe, frequency 23 kHz, amplitude 18 μm, 0–5°C) in the buffer described above. In preliminary experiments, cell lysis was confirmed by penetration of Evans' blue dye. All operations were carried out below 5°C. Protein content of intracellular samples was determined by the method of Bradford (1976) using the Bio-Rad kit with BSA as standard.

XET Assay

XET activity was determined by a method similar to that of Fry et al. (1992b). Reaction mixture (20 μL) containing 2 mg/mL *Tropaeolum* xyloglucan, 5.6 kBq [^{3}H]XG7-ol, and 50 mM phthalate (Na^+), pH 5.5, was mixed with 10 μL of extract. Incubation was for 1 h at 25°C, and the reaction was stopped with 100 μL of 20% formic acid. The solution was dried onto filter paper, which was then washed in tap water for 1 h to remove unreacted [^{3}H]XG7-ol, and then redried and assayed for radioactivity in 2 mL of toluene containing 0.5% PPO and 0.05% POPOP (counting efficiency for ^{3}H product was about 42%). Results are expressed as cpm incorporated into polymeric xyloglucan per hour. Preliminary kinetic experiments confirmed that a 1-h incubation period corresponded to the initial linear rate of reaction, which was proportional to the amount of extract added to the reaction mixture (see Fry et al., 1992b).

RESULTS

Cell Elongation

For 4 d after a 100-fold dilution into auxin-free medium, cell numbers in the Oxford cell line remained relatively constant, whereas packed cell volume showed about an 8-fold linear increase (Fig. 1a). Microscopy revealed that substantial cell elongation had occurred, with 80 to 90% of cells having changed shape by day 4. In contrast, after 10-fold dilution into standard (2,4-D-containing) medium, both packed cell volume and cell number increased linearly; a relatively constant ratio was maintained between these two parameters for at least 4 d (Fig. 1b), and the absence of any substantial cell elongation was verified microscopically.

Accumulation of XET activity in the medium was much more rapid in the elongating cells than in the nonelongating controls (Fig. 2), despite the much-lower cell density of the elongating cultures. Intracellular XET activity per mL of suspension (Fig. 2) remained relatively constant in both cultures.

The specific activity of intracellular XET increased progres-

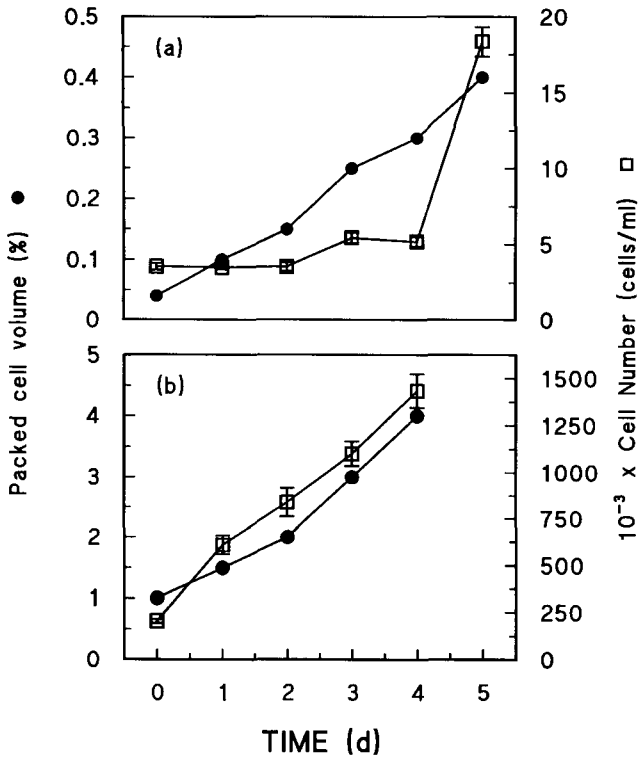

Figure 1. Growth of elongating and control carrot cell cultures. Packed cell volume (●) and cell numbers (□) in carrot cell suspensions (Oxford line) after (a) 100-fold dilution into auxin-free medium (elongating cells) or (b) 10-fold dilution into auxin-containing medium (nonelongating control cells). Mean values ± SE, $n = 6$.

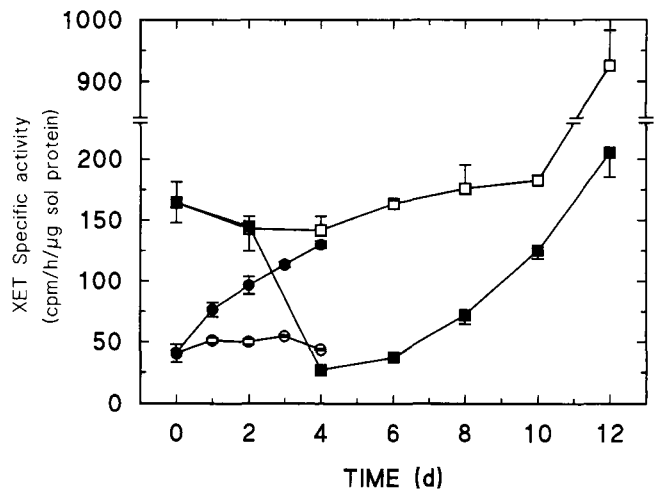

Figure 3. Specific activity of intracellular XET during elongation and embryogenesis. Oxford cell line elongating (●) and nonelongating control (○); line 10 embryo culture (■) and nonembryo control (□). Mean values ± SE, $n = 4$.

sively during elongation, but remained relatively constant in the nonelongating control culture (Fig. 3), despite the fact that much more XET was being liberated into the medium in the elongating culture. Thus, it appears that in the elongating culture, both the production of XET and its flux through the cell wall into the medium were enhanced.

Somatic Embryogenesis

XET activity accumulated to a high final concentration in the culture medium of the high-density suspensions used to maintain the embryogenic carrot cell line (line 10) (Fig. 4).

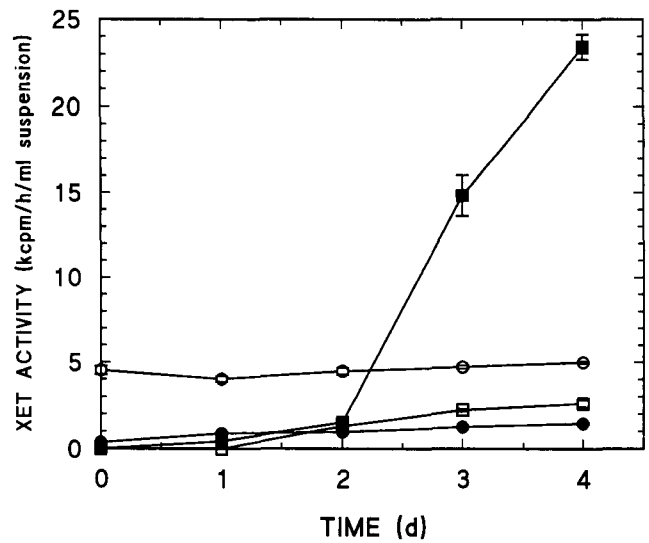

Figure 2. XET activity in elongating and control carrot cell cultures. Intracellular (○, ●) and extracellular (□, ■) XET activity in suspensions of elongating cells (●, ■) and nonelongating control cells (○, □). Oxford cell line, mean values ± SE, $n = 4$.

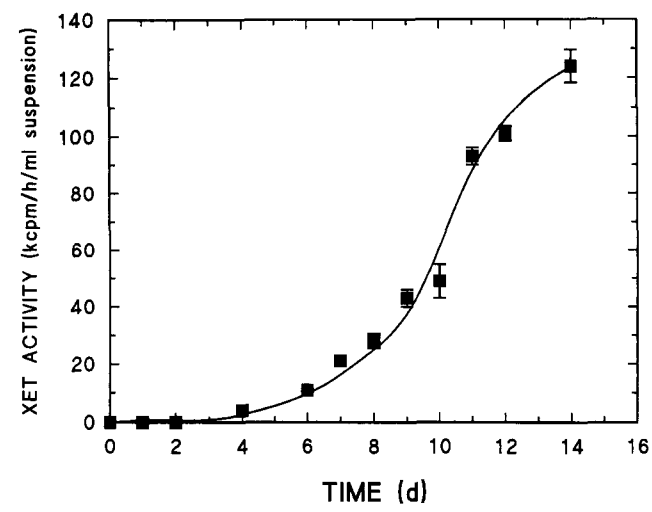

Figure 4. Accumulation of XET in the medium of line 10 under maintenance conditions. XET activity in the medium of an embryogenic high-density cell suspension (line 10) cultured at high cell density and in the presence of 2,4-D, thus preventing embryogenesis. Mean values ± SE, $n = 4$.

The profile of activity was sigmoidal over a typical 14-d subculturing cycle.

After dilution of a fraction enriched in PEMs into auxin-free medium, somatic embryogenesis proceeded asynchronously through recognizable developmental stages, namely globular, heart, torpedo, and cotyledonary embryoids (Fig. 5). When a PEM fraction was diluted to the same extent but into auxin-containing medium, no somatic embryogenesis occurred; microscopy revealed that this culture changed little over the 12-d period, with no obvious cell elongation or proliferation.

In the cultures undergoing embryogenesis, activity of both intracellular and extracellular XET remained low and relatively constant over the first 6 d, during which globular embryoids were forming (Fig. 6). Specific activity of intracellular XET markedly declined over the first 4 d (Fig. 3). However, after 6 d, when embryogenesis became more advanced with the presence of heart, torpedo, and cotyledonary stages, there was a substantial increase in XET activity, both intracellularly and extracellularly (Fig. 6), as well as in intracellular specific activity (Fig. 3). Between d 8 and 12, ≥95% of the XET activity was extracellular.

In the control cultures not undergoing embryogenesis, XET behaved differently. Extracellular XET was undetectable for 10 d, and even after 12 d activity was very low (Fig. 6). The specific activity of intracellular XET was relatively constant for 10 d, but increased markedly on d 12 (Fig. 3).

DISCUSSION

Xyloglucan is subject in vivo to both endohydrolysis (by cellulase) and endotransglycosylation (by XET). Carrot cell suspensions contained considerable XET activity; however, preliminary viscometric assays for cellulase (carboxymethylcellulase) showed no appreciable activity in either the spent medium or the cell extracts (data not shown). Thus, transglycosylation may have a much greater role to play in the cell wall than was previously thought. Bearing in mind the extraprotoplasmic nature of the enzyme (Nishitani and Tominaga, 1992), its mode of action (Smith and Fry, 1991), and the presence of higher activity in rapidly growing tissues (Fry et al., 1992b), we have proposed a role for XET in wall loosening (Fry et al., 1992a). We have now explored this idea in the context of cell elongation and somatic embryogenesis—two processes where alterations in cell size and shape, and therefore probably in wall properties, are important.

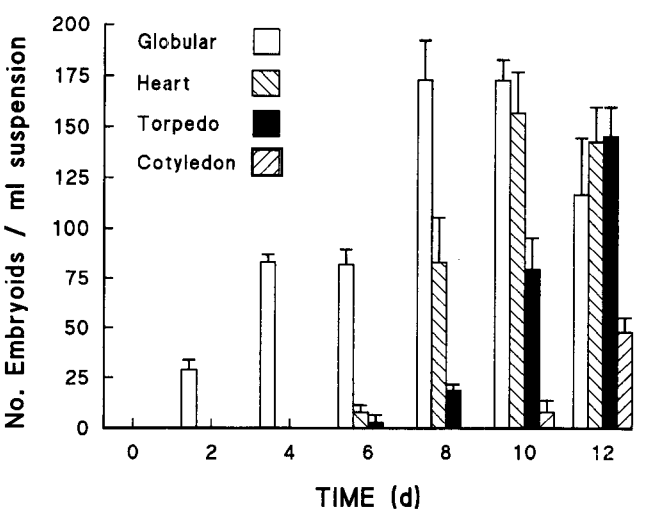

Figure 5. Progress of embryogenesis in line 10. Number and type of embryoids found during somatic embryogenesis in line 10 after dilution of a PEM-enriched culture into auxin-free medium. Mean values ± SE, $n = 4$.

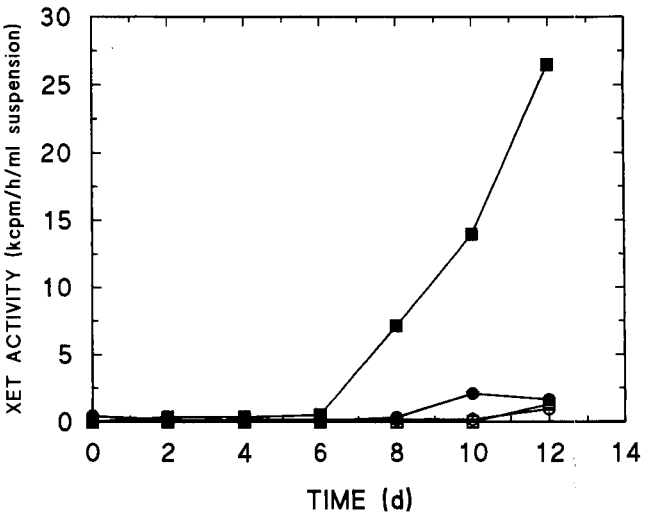

Figure 6. XET activity during embryogenesis. Intracellular (○, ●) and extracellular (□, ■) XET activities in line 10 carrot cells: embryo culture (●, ■), and nonembryo control (○, □). Mean values ± SE, $n = 4$.

The time courses for accumulation of XET activity in the medium, taken together with approximate cell numbers, indicate large differences between the various cell types in their rates of XET output per cell. Table I illustrates this for some representative sampling times. The Oxford cell line undergoing dramatic elongation in the virtual absence of cell division showed by far the highest rate of XET output (about 2000 microunits cell^{-1} d^{-1}). Moderate rates (about 6 microunits cell^{-1} d^{-1}) were seen in the high-density stock culture used to maintain line 10; this was a mixed population of cells including PEMs (showing negligible elongation), single elongating cells, and small aggregates of elongating cells. Low rates of XET output (≤2 microunits cell^{-1} d^{-1}) were seen in line 10 PEMs during the development of globular embryoids, in line 10 PEMs diluted into auxin-containing medium (the cells of which neither expanded nor divided), and in the nonelongating Oxford cell line. Thus, in these examples, there was an association between cell elongation and high rates of XET output per cell.

XET has been proposed to loosen the cell wall (Fry et al., 1992a), but it can only do so if it is in the right place. Very little XET activity appears to be bound to cell walls (Fry et al., 1992b); therefore, cell extracts will yield mainly intraprotoplasmic XET, which in vivo was not in contact with the wall and therefore was not capable of loosening it. Soluble

Table I. *Partitioning of XET between cells and medium in carrot cell cultures exhibiting various rates of cell elongation*

Cell Line	Condition of Culture	Elongation Rate	[XET] in Medium[a, b]	Total Rate of XET Output[a, b]	Cell Density[b]	Rate of XET Output per Cell[a]	Proportion of XET in Cell Extract[c]
			units mL^{-1}	*units mL^{-1} d^{-1}*	*mL^{-1}*	*microunits cell^{-1} d^{-1}*	%
Oxford	Elongating, day 3	++++	15	11	5.5×10^3	2000	7
Oxford	Non-elongating, day 3	−	2.2	2.3	1.1×10^6	2	68
10	Stock culture, day 10	±	60	28	$\sim 5 \times 10^6$	~6	N.D.
10	Globular embryos, day 4	−	0.33	0.06	$\sim 10^5$	~0.6	—
10	Mature embryos, day 10	+	14	4	$\sim 2.5 \times 10^6$	~1.6	13
10	No embryogenesis, day 4	−	<0.1	<0.02	2×10^4	<1	—
10	No embryogenesis, day 10	−	<0.1	<0.02	2×10^4	<1	—

[a] One unit is the amount of enzyme that catalyzes the production of 1 kcpm of ^{3}H-labeled product per hour. [b] mL refers to mL of spent culture medium. [c] $\left(\frac{\text{XET activity in cell extract}}{\text{XET activity in cell extract + XET in culture medium}}\right) \times 100\%$. N.D., Not determined; —, not detectable.

extracellular XET might be better placed to loosen the wall; however, the correlation between cell elongation and accumulated extracellular XET activity per mL of medium was poor (Table I), and different cells in the same flask elongated at different rates. (For example, the line 10 stock culture contained the highest extracellular XET concentration, yet most of the cells in this culture were not elongating.) By way of explanation, we suggest that soluble XET in the medium may not have ready access to the inner, load-bearing (Richmond, 1983) zone of the cell wall. We propose that any XET molecule is able to cause wall loosening for only the short period during which it is passing through the inner portion of the wall on its way from the plasma membrane into the culture medium. If this is so, the rate of XET secretion per cell would be the most meaningful parameter to consider, in accord with its good correlation with elongation (Table I).

Apparently in conflict with this interpretation, low rates of XET output per cell were also seen (Table I) in line 10 cultures that contained elongating (e.g. torpedo-shaped) embryoids (about 1.6 microunits cell^{-1} d^{-1} on d 10). However, since a torpedo-shaped embryoid is a large aggregate of small, closely packed cells surrounded by a cuticle, it would not be easy for XET molecules, secreted into the walls of internal tissues, to escape into the medium.

Very little XET was either accumulated in the cells or released into the medium during the formation of globular embryoids from PEMs. At this stage it is essential that the cells remain small and isodiametric (fail to elongate) for embryogenesis to proceed (Cordewener et al., 1991). After 6 d, globular embryoids developed into heart, torpedo, and cotyledonary stages, during which there is a requirement for localized cell elongation and expansion (Schiavone and Cooke, 1985) and therefore presumably wall loosening. During this stage, the specific activity of intracellular XET increased, suggesting a resumption of XET production, and some XET was also secreted into the medium.

Recent work has shown that elongation in particular cell types in an embryogenic high-density suspension can be inhibited by a lowering of the medium pH to 4 (Smith and Krikorian, 1992). This pH would be expected to inhibit substantially the activity of apoplastic XET (Fry et al., 1992b). However, although such high-density suspensions produce and secrete XET, they contain some cell types that do not elongate and remain small and isodiametric. This again is compatible with the idea that any XET molecule causes wall loosening only during its passage through the wall of the cell that secreted it.

ACKNOWLEDGMENTS

We thank Dr. S.C. De Vries, Agricultural University, Wageningen, The Netherlands, for cell line 10; Prof. K. Roberts, John Innes Institute, Norwich, UK, for the Oxford cell line; Prof. T.A. Truelsen, Århus University, Denmark, for providing a sample of pure tobacco cellulase used in preliminary experiments; Dr. E.P. Lorences, Valencia University, Spain, for purifying the XG7; and Miss Joyce Aitken for excellent technical assistance.

Received April 5, 1993; accepted July 15, 1993.
Copyright Clearance Center: 0032-0889/93/103/0987/06.

LITERATURE CITED

Baydoun EA-H, Fry SC (1989) *In vivo* degradation and extracellular polymer-binding of xyloglucan nonsaccharide, a natural anti-auxin. J Plant Physiol **134:** 453–459

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of proteins utilizing the principle of protein-dye binding. Anal Biochem **72:** 247–254

Cleland RE (1977) The control of cell enlargement. *In* Integration of Activity in the Higher Plant. Symposium XXXI. Society for Experimental Biology. Cambridge University Press, Cambridge, England, pp 101–115

Cordewener J, Booij H, Van Der Zandt H, Van Engelen F, Van Kammen A, De Vries S (1991) Tunicamycin-inhibited carrot somatic embryogenesis can be restored by secreted cationic peroxidase isoenzymes. Planta **184:** 478–486

De Vries SC, Booij H, Janssens R, Vogels R, Saris L, LoSchiavo F, Terzi M, Van Kammen A (1988) Carrot somatic embryogenesis depends on the phytohormone-controlled presence of correctly glycosylated extracellular proteins. Genes Dev **2:** 462–476

Fanutti C, Gidley MJ, Reid JSG (1993) Action of a pure xyloglucan *endo*-transglycosylase (formerly called xyloglucan-specific *endo*-(1→4)-β-D-glucanase) from the cotyledons of germinated nasturtium seeds. Plant J **3:** 691–700

Farkaš V, Sulová Z, Stratilova E, Hanna R, Maclachlan G (1992) Cleavage of xyloglucan by nasturtium seed xyloglucanase and transglycosylation to xyloglucan subunit oligosaccharides. Arch Biochem Biophys **298:** 365–370

Fry SC (1989) Cellulases, hemicelluloses and auxin-stimulated growth: a possible relationship. Physiol Plant **75:** 532–536

Fry SC, Smith RC, Hetherington PR, Potter I (1992a) Endotransglycosylation of xyloglucan: a role in wall yielding? Current Topics in Plant Biochemistry and Physiology **11:** 42–62

Fry SC, Smith RC, Renwick KF, Martin DJ, Hodge SK, Matthews KJ (1992b) Xyloglucan endotransglycosylase, a new wall-loosening enzyme activity from plants. Biochem J **282:** 821–828

Gamborg OL, Miller RA, Ojima K (1968) Plant cell cultures. Exp Cell Res **50:** 151–158

Gilkes NR, Hall MA (1977) The hormonal control of cell wall turnover in *Pisum sativum* L. New Phytol **78:** 1–15

Hayashi T, Maclachlan G (1984) Pea xyloglucan and cellulose. I. Macromolecular organization. Plant Physiol **75:** 596–604

Hayashi T, Wong Y, Maclachlan G (1984) Pea xyloglucan and cellulose. II. Partial hydrolysis by pea endo-1,4-β-glucanases. Plant Physiol **75:** 605–610

Hoson T, Masuda Y (1987) Effect of lectins on auxin-induced elongation and wall loosening in oat coleoptile and azuki bean epicotyl segments. Physiol Plant **71:** 1–8

Hoson T, Sone Y, Misaki A (1991) Xyloglucan antibodies inhibit auxin-induced elongation and cell wall loosening of azuki bean epicotyls but not oat coleoptiles. Plant Physiol **96:** 551–557

Knox JP, Linstead PJ, Peart J, Cooper C, Roberts K (1991) Developmentally regulated epitopes of cell surface arabinogalactan proteins and their relation to root tissue pattern formation. Plant J **1:** 317–326

Labavitch JM, Ray PM (1974) Turnover of cell wall polysaccharides in elongating pea stem segments. Plant Physiol **53:** 669–673

Lorences EP, Fry SC (1993) Xyloglucan oligosaccharides with at least two α-D-xylose residues act as acceptor substrates for xyloglucan endotransglycosylase and promote the depolymerization of xyloglucan. Physiol Plant **88:** 105–112

Lorences EP, Zarra I (1987) Auxin-induced growth in hypocotyl segments of *Pinus pinaster* Aiton: changes in molecular weight distribution of hemicellulosic polysaccharides. J Exp Bot **38:** 960–967

McCann MC, Wells B, Roberts K (1990) Direct visualization of cross-links in the primary plant cell wall. J Cell Sci **96:** 323–334

Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol Plant **15:** 473–497

Nishitani K, Tominaga R (1992) Endo-xyloglucan transferase, a novel class of glycosyltransferase that catalyzes transfer of a segment of xyloglucan molecule to another xyloglucan molecule. J Biol Chem **267:** 21058–21064

Rao PS (1959) Tamarind. *In* RL Whistler, ed, Industrial Gums. Academic Press, New York, pp 461–504

Richmond PA (1983) Patterns of cellulose microfibril deposition and arrangement in *Nitella*. *In vivo* analysis by a birefringence index. Journal of Applied Polymer Science: Applied Polymer Symposia **37:** 107–122

Schiavone FM, Cooke TJ (1985) A geometric analysis of somatic embryogenesis in carrot cell cultures. Can J Bot **63:** 1573–1578

Smith DL, Krikorian AD (1992) Low external pH prevents cell elongation but not multiplication of embryogenic carrot cells. Physiol Plant **84:** 495–501

Smith RC, Fry SC (1991) Endotransglycosylation of xyloglucans in plant cell suspension cultures. Biochem J **279:** 529–535

Taiz L (1984) Plant cell expansion: regulation of mechanical properties. Annu Rev Plant Physiol **35:** 585–657

Terry ME, Jones RL, Bonner BA (1981) Soluble cell wall polysaccharides released from pea stems by centrifugation. I. Effect of auxin. Plant Physiol **68:** 531–537

Widholm JM (1972) The use of fluorescein diacetate and phenosafranine for determining viability of cultured plant cells. Stain Technol **47:** 189–194