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Cell Wall Metabolism in Ripening Fruit[1]

VII. Biologically Active Pectin Oligomers in Ripening Tomato (*Lycopersicon esculentum* Mill.) Fruits

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A water-soluble, ethanol-insoluble extract of autolytically inactive tomato (*Lycopersicon esculentum* Mill.) pericarp tissue contains a series of galacturonic acid-containing (pectic) oligosaccharides that will elicit a transient increase in ethylene biosynthesis when applied to pericarp discs cut from mature green fruit. The concentration of these oligosaccharides in extracts (2.2 μg/g fresh weight) is in excess of that required to promote ethylene synthesis. Oligomers in extracts of ripening fruits were partially purified by preparative high-performance liquid chromatography, and their compositions are described. Pectins were extracted from cell walls prepared from mature green fruit using chelator and Na_2CO_3 solutions. These pectins are not active in eliciting ethylene synthesis. However, treatment of the Na_2CO_3-soluble, but not the chelator-soluble, pectin with pure tomato polygalacturonase 1 generates oligomers that are similar to those extracted from ripening fruit (according to high-performance liquid chromatography analysis) and are active as elicitors. The possibility that pectin-derived oligomers are endogenous regulators of ripening is discussed.

Work of the last 20 years has shown that many aspects of plant development can be influenced by application of cell wall-derived carbohydrates (Ryan and Farmer, 1991). Fruit ripening has received a considerable amount of attention in this regard (Baldwin and Pressey, 1988; Brecht and Huber, 1988; Tong and Gross, 1990; Campbell and Labavitch, 1991b), probably because cell wall digestion is such a prominent feature of this process (Huber, 1983; Fischer and Bennett, 1991). Most studies showing an effect of carbohydrates on ripening have utilized the tomato (*Lycopersicon esculentum* Mill.) fruit. Active fractions have been extracted from fruits and added to immature tissues, which then display aspects of ripening in advance of controls.

Oligosaccharides produced during the digestion of pectins have been shown to be active in many developmental systems (Ryan and Farmer, 1991), and this is certainly true for tomato ripening (Baldwin and Pressey, 1988; Brecht and Huber, 1988; Campbell and Labavitch, 1991a). This is not surprising, since the production of PG is a prominent early feature of ripening in tomatoes (Fischer and Bennett, 1991). If pectin fragments are a part of the endogenous regulation of ripening, then they must be present as ripening begins or proceeds, and this has not been convincingly demonstrated. Huber and Lee (1988) have demonstrated the in vitro production of pectic oligomers that are apparently (Brecht and Huber, 1988) able to promote tomato ripening, but the presence in vivo of active fractions is less clear. Huber and O'Donoghue (1993), using conventional chromatographic techniques, found no pectin oligomers in extracts of cell walls from ripening tomato fruits prepared under conditions that inactivated PG. The present work presents a contrasting result. Extracts of ripening tomato pericarp that had been pretreated with hot ethanol to inactivate PG have been analyzed by a sensitive HPLC approach and display a family of oligosaccharides that are apparently pectin derived. These were shown to be active in promoting the short-term production of ethylene of pericarp discs cut from MG tomatoes. Subsequent in vitro studies demonstrated the possibility that PG1 action on tomato cell wall pectins can generate active oligomers. The possibility of the involvement of these oligomers in the regulation of tomato ripening is discussed.

MATERIALS AND METHODS

Tomato (*Lycopersicon esculentum* Mill. var Castlemart) fruits were collected from vines grown in the fields at the University of California, Davis. MG fruits used for bioassays were at MG3 as described by Campbell and Labavitch (1991b). B fruits used for extraction had just begun to ripen as judged by the appearance of red color at the fruit's blossom end. Green fruits not used immediately were held at 10°C.

Oligomer Preparation

Mixtures of citrus pectin oligomers were prepared as described by Campbell and Labavitch (1991a). Mixtures of smaller (G7) and larger (G12) oligomers were used in various

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Abbreviations: B, breaker stage of tomato ripeness; CDTA, *trans*-1,2-diaminocyclohexane-*N,N,N',N'*-tetraacetic acid; G7 and G12, mixtures of shorter and longer, respectively, oligomers generated by partial acid hydrolysis of citrus pectin; MG, mature green stage of tomato ripeness; PAD, pulsed amperometric detector; PG, polygalacturonase; PGA, polygalacturonic acid; R, ripe stage of tomato ripeness.

treatments. When individual oligomers were needed the G7 and G12 preparations were subjected to QAE-Sephadex ion-exchange chromatography in an imidazole-HCl gradient, and fractions representing the centers of individual uronic acid peaks were pooled. Pooled material was desalted and concentrated by ultrafiltration through a YCO5 (Amicon Co., Beverly, MA) membrane (mol wt 500 cutoff). Although this procedure allowed some loss of smaller oligomers and did not remove all of the column buffer, it proved to be sufficient for our purposes, since controls (below) in assays of biological activity showed no impact of residual solutes.

Tomato oligomers were extracted from B fruits. Small sections of pericarp from unblemished fruits were cut into boiling 95% (v/v) ethanol and refluxed for 15 min. Ethanol was decanted and the tissue was homogenized in cold water using a Polytron (Brinkmann Instruments, Westbury, NY). After the sample was centrifuged (680*g*, 20 min) the supernatant was decanted and made 80% (v/v) in ethanol to precipitate oligomers. The pelleted material from the cold-water homogenate (crude cell wall extract) was used for pectin extraction.

We show (see below) that tomato PG action on cell wall pectins (in vitro) can generate biologically active pectin oligomers. It was important, therefore, to demonstrate that the oligomeric material isolated from ethanol-refluxed pericarp was not generated, following homogenization, by residual active PG. Although absolute proof of the absence of PG is difficult to provide, the point was tested in two ways. Portions of refluxed pericarp were homogenized in cold (4°C) 0.1 M Na acetate (pH 5.5) containing 1.7 M NaCl, 15 mM EDTA, and 5 mM 2-mercaptoethanol. After the tissue slurry was stirred in the cold for 3 h, the soluble fraction was collected by centrifugation (20 min at 12,000*g*) and made 80% saturated with $(NH_4)_2SO_4$. This suspension was stirred in the cold overnight, and insoluble material was collected by centrifugation. Pelleted material was dissolved in 10 mM Na acetate containing 100 mM NaCl and 1 mM DTT and dialyzed at 4°C against the same buffer. Aliquots of the dialyzed preparation were incubated with PGA, and the generation of reducing sugars (Gross, 1982) was assessed. Because incubations were lengthy (up to 48 h), a drop of toluene was added to reaction mixtures. A positive control (extract of nonrefluxed, red-ripe pericarp) was included with the test. No production of reducing sugars was measured during the incubation, whereas the production by extracts of untreated, ripe pericarp was off scale. Because PG could be tightly associated with cell wall components and thus protected against inactivation during refluxing, the autolytic capacity of the crude cell wall extract from ethanol-refluxed B pericarp was also tested. Walls were suspended in 0.1 M Na acetate (pH 5.0) at 37°C. There was no production of reducing groups during 24 h.The material precipitated from the supernatant in 80% ethanol was collected by low-speed centrifugation, dissolved in water, and applied to a column (11 × 100 mm) of QAE-Sephadex that had been equilibrated in 200 mM imidazole-HCl buffer (pH 7.0). After the column was washed with 200 mM buffer, acidic materials adsorbed on the column were eluted with 1.5 M imidazole-HCl. The bound fraction was dialyzed against cold H_2O (mol wt 1000 cutoff) and then concentrated by ultrafiltration, as described above. This procedure led to some loss of low mol wt materials. Extracts were also analyzed by gradient elution (125 mM to 1.5 M imidazole-HCl) of the QAE-Sephadex column.

HPLC Analysis

Analysis of oligomer distribution was carried out using a Dionex HPLC system with a 4 × 250 mm Carbopac PA-1 column and PAD. A gradient of Na acetate (400–900 mM) containing 100 mM NaOH was run at a flow rate of 0.8 mL/min over 45 min. When heterogeneity of purified individual oligomers was being assessed the same gradient was extended over 60 min. Peaks were detected with a PAD and integrated with a Chrom-Jet integrator (Spectra Physics, San Jose, CA). Quantification of individual oligomer peaks was based on calibrations using a galacturonic acid octomer that was almost homogeneous (i.e. greater than 90% of the integrated peak area; a gift from the Complex Carbohydrate Research Center, Athens, GA; Spiro et al., 1993). Preparative scale HPLC purification of individual tomato oligomer peaks was performed using the same gradient with a flow rate of 2.8 mL/min over 90 min and a Carbopac PA-1 column with dimensions of 35 × 280 mm. Peak fractions were collected, dialyzed against distilled water (as described above), and lyophilized.

Carbohydrate Analysis

Uronic acids in samples were measured according to the method of Blumenkrantz and Asboe-Hansen (1973) with galacturonic acid used as a standard. Samples were also analyzed by GC. Monomeric methyl glycosides were generated by methanolysis (Bhat et al., 1991) and subsequently converted into alditol acetates according to the method developed by Albersheim et al. (1967), as modified by Blakeney et al. (1983). Oligomeric materials were treated with methanolic HCl, reduced with $NaBH_4$, and acetylated with acetic anhydride in the presence of 1-methylimidazole. Elimination of the reduction step ($NaBH_4$) after methanolysis was used to quantify galactitol acetates that were derived from Gal (rather than galacturonic acid). Chromatography was accomplished using a 30 m × 0.25 mm DB-23 capillary column, operated isothermally at 210°C, using H_2 as the carrier gas. A Perkin-Elmer (Sigma 10) data system was used for quantification.

Cell Wall Preparation/Extraction/Digestion

The crude cell wall from the B pericarp homogenization step (above) was washed in 80% ethanol and then in acetone until colorless. It was dried in a vacuum oven. The dried cell wall material was extracted for 12 h at room temperature in 50 mM Na acetate (pH 6.5) containing 50 mM CDTA. The suspension was centrifuged for 15 min at 10,000*g* and the CDTA-soluble fraction was decanted. Two distilled water washes (resuspension of pellet followed by centrifugation) were added to the original supernatant, and this was dialyzed for 48 h at 4°C against several changes of distilled H_2O. The pellet was extracted in 50 mM Na_2CO_3 containing 20 mM $NaBH_4$ (12 h at room temperature). The Na_2CO_3-soluble fraction was collected and combined with two H_2O washes

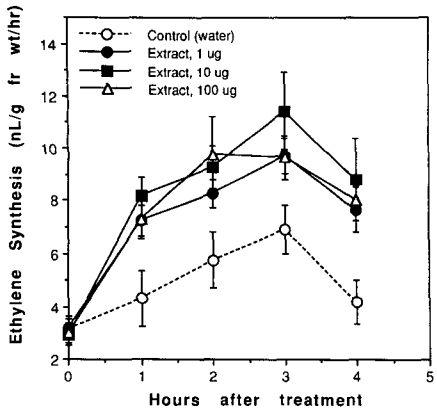

Figure 1. Promotion of ethylene synthesis in MG pericarp discs following treatment with water or varying concentrations (uronic acid equivalents) of B extract. Bars indicate SES for the means of measurements of eight pericarp discs per treatment. fr wt, Fresh weight.

of the pellet and dialyzed (as above). The dialyzed extracts were lyophilized.

Equal amounts (based on uronic acid equivalents) of the dried CDTA- and Na_2CO_3-soluble fractions and PGA (Sigma) were dissolved in 50 mM Na acetate (pH 4.5) and incubated at 37°C with purified tomato PG1 (a gift from A. Bennett, University of California, Davis). Aliquots were taken at intervals during the 48-h incubation period, the reaction was terminated by addition of cold 100 mM Na borate (pH 9.0), and reducing groups were measured spectrophotometrically using 2-cyanoacetamide (Gross, 1982). Oligomers in reaction mixtures were also assayed by analytical HPLC. In this case the reaction was terminated by heating (100°C) the mixture for several minutes. The heat-inactivated mixtures were also used for assessment of biological activity.

Biological Assay of Ethylene Induction

The model system for the bioassay was the tomato pericarp disc system of Campbell et al. (1990). Discs cut from the outer pericarp of MG tomatoes were approximately 12 mm in diameter, 4 mm in thickness, and 350 mg in weight. Treatments were given in 10 μL of solution applied with a micropipette in several droplets over the disc's cut inner surface. Treatment amounts refer to the amount of material applied per disc. Control treatments were also of 10 μL. Ethylene production following treatment was measured on individual discs by GC as described by Campbell et al. (1990).

RESULTS AND DISCUSSION

In the preliminary tests, aliquots of the water-soluble, ethanol-insoluble fraction of B tomato pericarp homogenates containing 50 μg of uronic acid equivalents promoted short-term ethylene synthesis when applied to MG pericarp discs (not shown). The response was similar to that caused by addition of a similar amount of a mixture of acidic oligomers produced by partial acid hydrolysis of citrus pectin. In separate tests, a range of concentrations of the B extract was applied to discs. The promotion of ethylene synthesis (Fig. 1) was near maximum with a 1-μg (galacturonic acid equivalents) treatment of the 350-mg pericarp discs.

Following an initial anion-exchange step to remove neutral sugars from the B extract, both the B fruit extract and the citrus pectin oligomer mixtures were subjected to HPLC ion-exchange separation in an alkaline Na acetate gradient (Fig. 2). Because the separation is on the basis of charge properties of the component molecules rather than strictly on the basis of molecular size, we are unable to make a strict physical comparison of the tomato and citrus pectin preparations. Major peaks in the two citrus preparations often have slightly different retention times and the separation pattern for the tomato extract matches neither of the citrus preparations. All patterns, however, suggest a series of similar acidic species having regular differences in charge properties. For each sample the baseline increases slightly with increasing retention time, suggesting the presence of larger species that become less well resolved. This is similar to the separations shown by Hotchkiss and Hicks (1990).

We have observed a decreasing sensitivity of the PAD with neutral dextrans of increasing mol wt; therefore, it would be incorrect to assume that peak areas strictly indicate the proportions of various members of a given series. Rough esti-

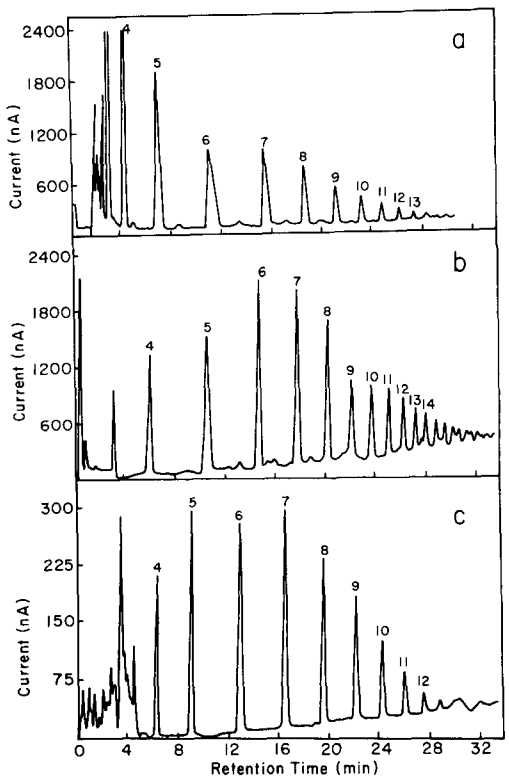

Figure 2. HPLC ion-exchange gradient separation of samples (25 μg of uronic acid equivalents each) of smaller (G7) citrus pectin oligomers (a), larger (G12) citrus pectin oligomers (b), and B extract (c). Detection was by PAD. For each sample the peak co-eluting with the galacturonic acid octomer was designated peak 8, and the remaining peaks were numbered consecutively from peak 8.

Table I. *Distribution of uronic acids in acid oligomers resolved by HPLC gradient ion-exchange chromatography (Fig. 2)*

Quantitation is based on comparison of integrated areas with the peak area for a known amount of galacturonic acid octomer and is subject to the uncertainties discussed in the text. Samples (25 μg, galacturonic acid equivalents) of G7, G12, and B extract were chromotographed. Yield is the sum of the uronic acid measured in all oligomer peaks for a given sample expressed as a percentage of the 25 μg injected. Oligomer peak numbers are shown in Figure 2 and the values shown are in ng of galacturonic acid equivalents.

Sample	Peak No.											Yield
	4	5	6	7	8	9	10	11	12	13	14	
G7	902	1190	694	485	347	274	232	201	104	90		18.00
G12	462	563	619	614	463	341	295	286	189	129	83	16.70
B extract	50	80	93	106	67	42	26	23	12			2.00

mates of the concentrations of the oligomer populations (Table I) in the citrus preparations and the B extract were obtained by comparisons of integrated peaks with the area measured following injection of a known amount of galacturonic acid octomer. The total concentration of acid oligomers in the B extract represented by peaks eluting between 5 and 26 min (Fig. 2c) represents approximately 2.2 μg/g fresh weight of tomato pericarp. Acid oligomers measured in the G7 and G12 mixtures produced by partial hydrolysis of citrus pectin represented a substantially greater proportion of the uronic acid subjected to HPLC than did the B extract (Table I). These figures can be taken only as estimates because of the lack of strict linearity in PAD response and because peaks in the extracts are not exclusively (if at all) homooligomers (Table II). The analysis also indicates that oligomers account for less than 2% of the total uronic acid in the B extract. This conclusion is consistent with the results of gradient elution QAE-Sephadex chromatography of the B extract (Fig. 3). Groups of uronic acid-containing fractions were pooled, concentrated, desalted using a YC05 membrane, and analyzed by HPLC. Acidic oligomers were found early in the separation (pooled fractions 26–41; Fig. 3) and not in pools representing the larger uronic acid peaks (see legend to Fig. 3). Thus, if the promotion of ethylene synthesis by the B extract is due to its acidic oligomer content, then the response of a 350-mg disc to 10 μg of extracted uronic acid (Fig. 1) represents a response to 200 ng of oligomers. This is easily within the concentration present in B fruits.

Because biological activity could be due to larger, unresolved molecules rather than those cleanly separated in the oligomeric series, we isolated the material in individual peaks and subjected these to assay of biological activity. For the citrus preparation with the shorter average degree of polymerization we used the protocol of Campbell and Labavitch (1991a) and collected peak fractions from a standard QAE-Sephadex gradient separation (Fig. 4a). Subsequent HPLC analysis (Fig. 4b) showed that the QAE-Sephadex separation provided a relatively effective purification step. The concentration of oligomeric material in fruit extracts was too low to permit efficient colorimetric detection of individual oligomer peaks following a QAE separation (Fig. 3), although peaks in samples enriched in oligomers (e.g. G7 and G12) can be resolved by the QAE analysis (Fig. 4). Therefore, purification was by preparative scale HPLC combined with collection of PAD-identified fractions. Neutralized fractions from the QAE and HPLC purifications were desalted and tested for elicita-

Table II. *Carbohydrate compositions (weight percentage) of individual oligomer peaks purified (QAE-Sephadex or HPLC ion-exchange separation, respectively) from mixtures of citrus pectin oligomers or B fruit extracts*

Compositions shown are for peaks whose biological activity is described in Figure 5. Uronic acid values are based on colorimetric assay. Proportions of neutral sugars were determined by GC and adjusted so that totals equal 100%. In fact, some oligomers (G7: peaks 8, 9 and 10. B extract: peak 10) produced small (less than 1% of the total integrated area), unknown peaks in the GC chromatograms.

Source	Component						
	Galacturonic acid	Rha	Ara	Xyl	Man	Gal	Glc
Citrus oligomers							
Peak 8	93.9	1.1	2.0	0.2	0.3	1.0	1.4
Peak 9	95.5	0.9	1.9	0.1	0.2	0.5	0.8
Peak 10	86.7	2.5	3.3	0.8	1.7	1.9	3.2
B extract							
Peak 7	71.1	6.2	3.3	1.2	4.5	4.6	9.1
Peak 8	58.2	5.8	3.1	1.8	2.4	15.0	13.8
Peak 9	55.1	13.2	4.4	2.6	5.0	11.8	7.9
Peak 10	56.4	10.1	5.9	1.5	2.9	18.5	4.7
Peak 11	60.8	3.3	3.3	1.9	3.5	14.5	12.8

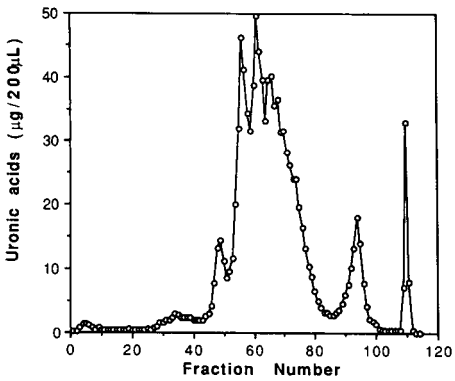

Figure 3. QAE-Sephadex gradient separation of the B extract. Sample (18 mg of uronic acid equivalents in 20 mL of 125 mM imidazole-HCl [pH 7.0]) was applied to the column (11 × 100-mm gel bed). The column was then eluted with 50 mL of 125 mM buffer, followed by a 125 mM to 1.5 M buffer gradient (500 mL), and finally 50 mL of 1.5 M buffer. Fractions of 5 mL were collected, and aliquots were assayed for uronic acids. Groups of fractions (26–41, 45–50, 53–75, and 84–100) were pooled, concentrated by ultrafiltration, and analyzed for oligomers by HPLC.

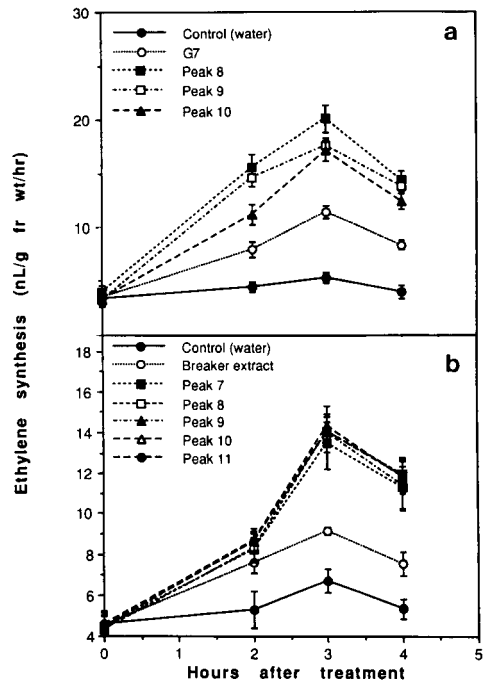

Figure 5. Ethylene production by MG pericarp discs treated with 50 μg (uronic acid equivalents) of purified G7 citrus oligomers (a) or 30 μg of purified endogenous pectin oligomers (b). a, Water is the control. Treatment is with the G7 mixture or individual oligomers from the QAE-Sephadex separation shown in Figure 4a. b, Treatment is with the unfractionated B oligomer mixture or individual peaks purified by preparative HPLC (peak 7 is that shown eluting at approximately 16 min in Fig. 2c). Bars indicate SES for the means of measurements of eight pericarp discs per treatment.

tion of short-term ethylene synthesis (Fig. 5). Not all peaks of the two preparations were tested, but it is clear that separated oligomer peaks are more active than equal amounts (uronic acid equivalents) of the mixture. Resolution (by either QAE or HPLC gradient) of oligomeric peaks decreases with increasing size/elution time, so peaks assigned higher numbers should be assumed to be less pure.

Carbohydrate analysis (Table II) of the better-resolved peaks from the two preparations indicates that homooligomers of galacturonic acids are either not present or do not make up the entirety of any isolated peak material. A substantial neutral sugar component is found in each of the peaks from tomato fruits. If homooligomers of galacturonic acids are present in these preparations they are definitely a minor component.

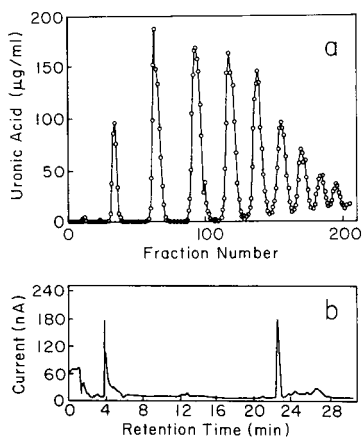

Figure 4. a, QAE-Sephadex ion-exchange separation (column dimensions: 14 × 200 mm) of G7 citrus pectin oligomers in a pH 7.0 imidazole-HCl buffer (0.2–0.9 M in 1000 mL) gradient. Fractions of 4 mL were collected and aliquots assayed for uronic acids. Uronic acid oligomers pooled and concentrated by ultrafiltration for use in assays (Fig. 5a) as peaks 8, 9, and 10 were fractions 168 to 174, 181 to 187, and 193 to 197, respectively. b, HPLC ion-exchange analysis of 500 ng (uronic acid equivalents) of peak 8 from the QAE separation shown in a. Detection was by PAD. Peak 8 co-chromatographs with the galacturonic acid octomer.

These results show that oligomeric materials, perhaps derived from a complex cell wall pectin, are present in tomato fruits at B and, thus, must be considered to be potential modulators of ethylene synthesis. Campbell and Labavitch (1991b) have shown that citrus pectin-derived oligomers can promote the ripening of MG pericarp discs (long-term response) as well as a short-term increase in ethylene synthesis such as that shown here (Figs. 1 and 5b). Whether the endogenous oligomers described herein can also promote ripening must await further testing. And, of course, if endogenous oligomers are to be considered endogenous regulators of ripening, they must be shown to be present in MG discs at the time ripening begins. We have preliminary data that are consistent with this. The small amounts of uronic acid that can be collected by centrifuging (Morrison et al., 1987) water-infiltrated pericarp discs were subjected to PAGE separation as described by Vreeland et al. (1990). A clear series of stained oligomer bands was seen in preparations from B and R discs, and a few weakly stained bands were also seen

in the preparation from MG discs (not shown). Biological activity of the MG extracts has not been tested.

A logical explanation for the appearance of pectic oligomers is that PG, whose activity increases substantially as tomato fruits ripen, hydrolyzes a wall pectin to produce oligomeric products. This possibility was tested by preparing CDTA-soluble (chelator-soluble) and Na_2CO_3-soluble pectins from cell walls of MG tomatoes and incubating these with purified tomato PG1. Surprisingly, only the Na_2CO_3-soluble material and PGA, the latter used as a positive control, were digested (reducing sugar assay, not shown). When the reaction mixtures were subjected to HPLC analysis a series of oligomeric products was observed in each (Fig. 6). The digested Na_2CO_3-soluble pectin was also active in promoting short-term ethylene production (Fig. 7), as was the control reaction mixture containing PG1 and commercial PGA. As time of incubation of PG1 with the Na_2CO_3-soluble pectin or PGA increased, the ethylene synthesis-promoting activity of the reaction mixture (based on uronic acid equivalents) first increased and then decreased (Melotto, 1992, and data not presented), suggesting that an intermediate size of oligomer is optimal for eliciting activity. The undigested substrates and the mixture of PG1 plus CDTA-soluble pectin were not active. Clearly, our in vitro active oligomer generation system is an artificial one. PG action on cell wall-localized substrates could be modulated by the presence of esterified uronosyl residues, pectin side chain and backbone modifications, and other steric factors. The alkaline Na_2CO_3 extractant would have saponified esters while solubilizing the wall pectin and thus could have converted a normally unsusceptible substrate into one that PG could hydrolyze. Carrington et al. (1993), however, compared cell wall changes in normal tomatoes and those lacking PG (antisense suppression) and showed that a primary substrate for PG is the tomato cell wall Na_2CO_3-soluble pectin.

Huber and O'Donoghue (1993) recently reported that they were unable to find pectin oligomers in extracts of tomato cell walls that had been prepared (Tris-buffered phenol; Huber, 1991) so as to eliminate residual PG activity. Our pericarp tissue preparations were first treated in boiling 95% ethanol for 15 min before homogenization/extraction in water. We found that this treatment provides cell walls that are autolytically inactive and from which no detectable PG activity can be extracted. We must recognize, nevertheless, that residual active PG could have contributed to the production of oligomers from solubilized substrates just as PG1 does when mixed with Na_2CO_3-soluble pectin. The pectin preparations that Huber and O'Donaghue assayed for oligomer presence would have been a combination of our water-soluble pectin and the chelator-soluble polymers that we have shown to be inactive in elicitation. Based on our analysis, the presence of oligomers in their extract would have been much less than the 2% (of uronic acids) that we have reported for water-soluble pectins alone. It is probable that their combination of colorimetric assay and conventional gel filtration chromatography was not sufficiently sensitive to resolve/detect oligomers present in low concentrations. This

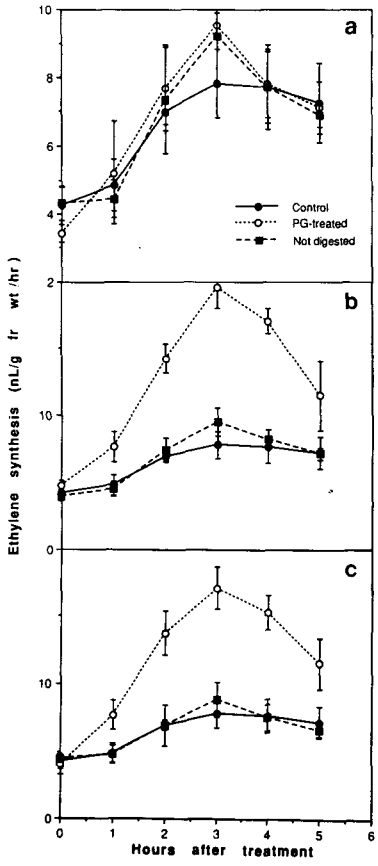

Figure 7. Effect of PG1-treated CDTA-soluble (a) or Na_2CO_3-soluble (b) MG pectins and commercial PGA (c) on ethylene production by MG pericarp discs. PG1-digested pectins were incubated for 3 h (HPLC profiles for digested PGA and Na_2CO_3-soluble pectins are shown in Fig. 6). Controls were treated with heat-denatured PG1 incubated without substrate. "Not digested" refers to tests with undigested substrates. Treatments were with 10 μg of uronic acid equivalents (controls received no uronic acid). Line legends shown in a apply to all three panels. Bars indicate SES for the means of measurements of eight pericarp discs. fr wt, Fresh weight.

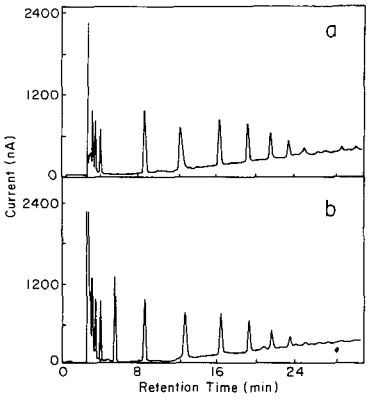

Figure 6. HPLC ion-exchange gradient separation of 25 μg (uronic acid equivalents) of PGA (a) or Na_2CO_3-soluble pectin from MG Castlemart tomato (b) that had been incubated at 37°C with purified tomato PG1 for 3 h at pH 4.5. Detection was by PAD.

is the same problem we had when attempting to resolve water-soluble oligomers in the B extract using QAE-Sephadex (Fig. 4).

Although our work with PG1 provides an explanation for the appearance of pectin oligomers in ripening tomato tissue, it tends to argue against a role for oligomers in the initiation of ripening. Several studies (Brady et al., 1982; Grierson et al., 1985) have shown that the appearance of PG protein and activity in extracts of tomato fruit tissues occurs after the ripening-associated increase in ethylene production. Thus, if pectic oligomers are to play a role in the initiation of ripening and are produced by the action of pectolytic enzymes, then the responsible enzyme (PG or some other pectolytic agent, e.g. rhamnogalacturonase [Schols et al., 1990]) must have been missed in prior investigations. Oligomers might be present in the pectins that are synthesized in fruits at the onset of ripening (Huysamer et al., 1992). Alternatively, oligomers might not originate in the fruit. (The demonstration that homooligomers of galacturonic acid are mobile in tomato plants [MacDougall et al., 1992] supports this possibility.) Of course, oligomers could play a role in the regulation of ripening without being involved in its initiation. The recent demonstration of an apoplastic localization for tomato fruit ACC oxidase (Rombaldi et al., 1994) may be relevant to the connection between cell wall breakdown and ethylene synthesis described in the present work. These, as well as other questions indicated above, are the subjects of continuing work.

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