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Endopolygalacturonase in Apples (*Malus domestica*) and Its Expression during Fruit Ripening[1]

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The activity of polygalacturonase (PG) has been detected in ripe McIntosh apples (*Malus domestica* Borkh. cv McIntosh) both by enzyme activity measurement and immunoblotting using an anti-tomato-PG antibody preparation. PG activity increased during fruit ripening and remained steady, or decreased slightly, after 5 months of controlled atmospheric storage. The enzyme had a relative molecular weight of 45,000 as determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and 56,000 to 61,000 when determined by gel filtration. Viscosity and reducing end group measurements with a commercial pectin preparation showed that the enzyme is endo acting. In RNA and DNA blot hybridization experiments, a full-length tomato PG cDNA hybridized with the apple RNA and DNA, showing the identity of genes encoding the activity of the enzyme in tomato and apple.

Structural changes in the cell walls of climacteric fruits that lead to softening of the tissues are initiated by the action of ethylene, the fruit-ripening hormone (Abeles, 1985). The softening of the fruit tissues is the consequence of cell wall-degrading enzymes (Grierson et al., 1985), of which endo-PG (EC 3.2.1.15) was reported to cause the most prominent changes (Crookes and Grierson, 1983). Endo-PG was implicated in tissue softening because its appearance during fruit ripening (DellaPenna et al., 1989) corresponds to the increase in fruit softening. In a number of cultivars, a correlation between PG activity and fruit softening has been observed (Crookes and Grierson, 1983). Also, application of isolated endo-PG preparations to fruit tissue discs resulted in ultrastructural changes that were similar to those occurring during the normal ripening process (Ben-Arie et al., 1979). Ripening mutants with delayed or decreased softening that were deficient in PG activity (DellaPenna et al., 1989; Koch and Nevins, 1990) gave further indications that the enzyme plays a major role in the softening of fruits.

Results of recent experiments with transgenic tomato fruits, however, are not in agreement with the above assessment. Fruit softening was not affected significantly in tomatoes with down-regulated PG activity (Giovannoni et al., 1989; Smith et al., 1990). The results of transgenic experiments suggest further that although PG activity causes depolymerization of polyuronides in fruits, the enzyme is not the sole cause of the cell wall structural changes during the softening process. Transgenic experiments with *rin* tomato mutants showed that when the expression of the PG gene has been up-regulated using the promoter region of another ripening-associated gene (Schuch et al., 1989), PG production and polyuronide degradation are increased without any detectable effect on fruit softening. These data suggest that although endo-PG activity increases during the ripening process of tomatoes, and the increased activity of this enzyme results in structural changes of the middle lamella, the softening of fruits is a more complex process than anticipated.

The ripening process of fruits has been best investigated in tomatoes; however, other fruits such as peaches (Lee et al., 1990), papayas (Lazan et al., 1989), and pears (Pressey and Avants, 1976) also showed elevated activity of an endo-PG during ripening. From biological considerations it is expected that the basis of the biochemical changes occurring during the ripening process is similar in most or all fruits, and that differences in the rate of ripening are due to differential rate of expression of the enzymes involved (i.e. quantitative instead of qualitative differences). Although endo-PG activity was reported in most fruits during the ripening process, apples were thought to be unique among fruits in expressing the activity only of an exo-acting PG (Kertesz, 1951; Pilnik and Voragen, 1970; Bartley, 1978; Abeles and Biles, 1991).

In this paper, we have reinvestigated the presence of and mode of action of a PG that is expressed during the ripening process. In the investigation, we have used the tools of biochemistry, immunology, and molecular biology. Our results clearly show the presence of an endo-PG in ripening apples.

MATERIALS AND METHODS

Plant Material

McIntosh apples (*Malus domestica* Borkh. cv McIntosh) were sampled at several stages of fruit development and ripening. Apples were peeled, and the exocarp and mesocarp tissues were frozen in liquid nitrogen and stored at −100°C until use.

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Abbreviations: PG, polygalacturonase; TBST, Tris-buffered saline containing 0.05% Tween-20.

The puncture and deformation testing of fruits was performed according to Bourne (Bourne, 1965). Thirty-three apples were used at each ripening stage. One punch was made with a 1.11-cm diameter pressure tip in each apple at the equator, with the pericarp removed from the area to be punctured. Deformation of fruit was tested in the same instrument using a flat plate with the deformation force of 1 kg instead of the penetration tip.

Assays

Anthocyanin content of apple exocarp tissue (2.0 g) of different ripening stages was determined as described previously (Wellmann et al., 1976). Chl was measured as described by Arnon (1949). Protein was determined by a micro method adopted from Bradford (1976).

Extraction and Partial Purification of PG

Apple exocarp (60–100 g) and mesocarp (200 g) and tomato exocarp (100 g) tissues were homogenized with 5% (w/w) polyvinylpolypyrrolidone in extraction buffer (DellaPenna et al., 1987) containing 5 mM β-mercaptoethanol, and the homogenate was squeezed through a fine-mesh nylon cloth. The pulp was resuspended in 1.7-M NaCl solution containing 15 mM EDTA and 50 mM sodium citrate (pH 5.5) and stirred for 1 h. The suspension was filtered through a double layer of fine-mesh nylon cloth and proteins were precipitated by ammonium sulfate in the 30 to 80% saturation range.

For the partial purification of the enzyme, apples (2 kg) were cored, frozen in liquid nitrogen, and homogenized in 2.5 L of extraction buffer containing 5% (w/v) polyvinylpolypyrrolidone (DellaPenna et al., 1987). The homogenate was pressed through a fine-mesh nylon cloth and the pulp was resuspended in 1 L of 1.7-M NaCl solution containing 15 mM EDTA and 50 mM sodium citrate (pH 5.5). The suspension was stirred for 3 h and filtered through a double layer of the fine-mesh nylon cloth. Protein was precipitated from the filtrate by the addition of 30% (w/v) solid ammonium sulfate and discarded. PG was precipitated by the addition of ammonium sulfate to 80% (w/v) saturation and collected by centrifugation at 20,000g for 20 min. The pellet was dissolved in 15 mL of sodium acetate buffer (pH 6.0) containing 100 mM NaCl and 1 mM DTT and dialyzed against the same buffer for 4 h. This preparation was loaded onto a Sephadex G-100 gel-filtration column (2.5 × 80 cm) and chromatographed using the same buffer. Four-milliliter fractions were collected and analyzed for protein content and PG activity.

Determination of Enzyme Activity

PG activity was measured by determining the production of reducing sugars using the arsenomolybdate method (Nelson, 1944). The reaction mixture consisted of 0.2 mL of enzyme preparation, 0.1 mL of 100 mM sodium acetate buffer, pH 4.5, 20 μL of 2 M NaCl, and 0.1 mL of 1% polygalacturonic acid (Sigma). Reaction mixtures were incubated at 37°C for 30 min. Reducing groups were assayed using α-galacturonic acid as standard. Viscosity of the pectin solution in the presence of a partially purified PG preparation was determined in a calibrated Cannon-Fenske Viscometer. The reaction mixture contained 0.24% (w/v) polygalacturonic acid, 1.8 mmol of NaCl, 0.36 mmol of sodium acetate, 6 μmol of DTT, and 660 μg of partially purified PG in a total volume of 12.6 mL.

TLC of the enzymic hydrolysis products was carried out on precoated silica gel plates (20 × 20 cm, Sigma). The reaction mixtures were boiled for 10 min to stop the enzymic reaction and the undegraded polygalacturonic acid was precipitated by addition of 4 volumes of ethanol. The precipitate was removed by centrifugation at 12,000g for 15 min, and the supernatants were lyophilized. The residue after lyophilization was dissolved in 200 μL of water. Eighty microliters of this solution was loaded on the TLC plates. The chromatograms were developed in n-butanol:formic acid:water (2:3:1) with galacturonic acid as standard and visualized with aniline-diphenylamine (Sigma) (Koller, 1966).

Electrophoresis and Immunoblotting

Electrophoresis of proteins was carried out in denaturing polyacrylamide gels (10%) as described by Laemmli (1970). Immunoblotting was carried out as described (Towbin et al., 1979). Protein samples (5 μg/lane for apple and 2 μg/lane for tomato) were loaded onto denaturing polyacrylamide gels (10%) and electrophoresed. Protein was electroblotted onto nitrocellulose membranes for 2 h at 150 mA per gel. The nitrocellulose membrane was blocked for 30 min in TBST buffer containing 0.4% Carnation nonfat dry milk and for 1.5 h in TBST buffer containing 5% Carnation nonfat dry milk. The blot was incubated overnight at room temperature with a rabbit anti-tomato PG antiserum (DellaPenna et al., 1986) (1:1000, diluted in TBST buffer containing 0.4% Carnation nonfat dry milk). The blot was washed three times with 100 mL of TBST for 45 min and incubated with a goat anti-rabbit immunoglobulin G-alkaline phosphatase conjugate (Sigma, 1:7500 dilution in TBST buffer). Visualization of the immunoconjugate was with 66 μL of nitroblue tetrazolium and 33 μL of 5-bromo-4-chloro-3-indolyl phosphate in 10 mL of alkaline phosphatase buffer.

RNA Extraction and Northern Blot Hybridization Analysis

Ten aliquots of freeze-dried apple tissue (4 g each) were ground to powder in liquid nitrogen using a mortar and pestle according to the procedure of Verwoerd et al. (1989). The powdered tissue aliquots were extracted with a 5-mL mixture of phenol:extraction buffer, 1:1 (100 mM LiCl, 100 mM Tris, pH 8.0, 10 mM EDTA, and 1% [w/v] SDS). Chloroform (2.5 mL/sample) was added to the extracts and the phases separated by centrifugation at 3,000g. The aqueous phase was removed and RNA was precipitated by the addition of 4 M LiCl and storage at −20°C for 1 h. The precipitated RNA was collected by centrifugation at 11,000g. Contaminating polysaccharides were removed from the preparation by the method of Fang et al. (1992). Total RNA was precipitated with 2.2 volumes of ethanol and 0.1 volume of 3 M sodium acetate at −20°C for 1 h, centrifuged, and dissolved in diethylpyrocarbonate-treated water. Poly(A)$^+$ RNA was selected from total RNA on Hybond-mAP (Amersham Inter-

national) following the manufacturer's instruction. Poly(A)$^+$ RNA (10 μg) was electrophoresed in a 1% formaldehyde denaturing agarose gel and blotted onto a GeneScreen Plus membrane (New England Nuclear Research Products). The membrane was prehybridized in a mixture of 0.75 M NaCl, 0.13 M sodium citrate, 0.6% SDS, 50 mM sodium phosphate, 5× Denhardt's solution, 2.5 mM EDTA, 5% dextran sulfate, and 100 μg of denatured calf thymus DNA at 65°C for 6 h and hybridized with a labeled cDNA insert of pPG1.9 containing the full-length tomato PG cDNA (DellaPenna et al., 1989) in the same buffer for 22 h at 65°C. The membrane was washed twice in a mixture of 0.3 M NaCl, 0.03 M sodium citrate, and 0.1% SDS (pH 7.0) at 60°C for 30 min and subjected to autoradiography at −90°C.

DNA Extraction and Southern Blot Hybridization Analysis

High mol wt DNA was extracted from expanding apple (cvs Robusta 5, Rome Beauty, and White Angel) leaf tissue using the method described by Saghai-Maroof et al. (1984). This DNA (15 μg per sample) was digested with *Eco*RI, *Hin*dIII, *Eco*RV, *Bgl*II, *Bam*HI, *Xba*I, and *Dra*I restriction enzymes, electrophoresed in a 1% agarose gel, blotted onto GeneScreen Plus, and probed with the nick-translated insert of pPG1.9 (DellaPenna et al., 1989). Prehybridization, hybridization, washing procedures, and autoradiography were carried out as described previously for the isolation of RNA.

RESULTS

Changes in Texture, Color, and PG Activity during Ripening of Apples

The softening of fruits during ripening may be determined by measuring the force required for a probe to penetrate into the mesocarp (penetrometer) and the deformation of the fruit upon application of constant force (Bourne, 1969). When McIntosh apples in different stages of maturation and ripening were subjected to penetrometric tests, a constant decrease in the puncture force was observed in the fruits with increasing degree of fruit development and ripening. Significant changes in deformability were observed only after the fruits reached maximum size (Fig. 1A). During the same time, between July 5 and August 17, the Chl content of apple fruits showed a sharp decrease and remained at a low level during the further stages of development and ripening (Fig. 1B). Anthocyanin accumulation in the exocarp commenced after August 17 and showed an increase until October 26, after which date freezing injury of the fruits prevented further sampling.

PG activity in the fruits was below the threshold of detectability until 6 weeks after fruit setting. Enzyme activity increased nearly linearly during the subsequent maturation and ripening of apples until freezing injury prevented further harvest. In fruits harvested on October 26 and stored for 5 months under controlled atmospheric conditions, there was a measurable decline in the activity of the enzyme (Fig. 1C). When protein preparations from exocarp cell wall extracts were subjected to electrophoresis and immunoblotting using the anti-tomato PG antibody, increased amounts of a protein band with the approximate M_r of 45,000 were observable during the maturation and ripening stages of the fruits (Fig. 1D).

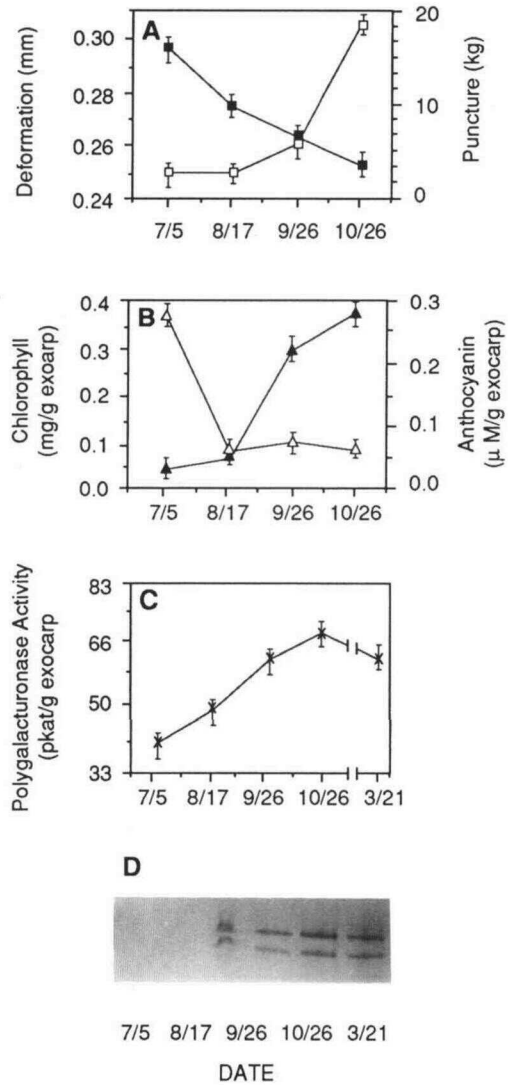

Figure 1. Changes in deformation (□) and puncture force (■) (A), Chl (△) and anthocyanin (▲) (B), PG activity (C), and specific PG protein (D) during development and ripening of McIntosh apples. D shows an immunoblot from the same samples as used for the determination of PG activity in C. Datapoints in A, B, and C represent an average of three experiments. SE is shown by bars.

Enzymological, Immunological, and Molecular Characterization of PG

Apple fruits contain relatively low amounts of protein as compared with tomatoes (Table I). Whereas tomato exocarp tissues contained approximately 190 μg of protein/g fresh weight, the protein content of apple exocarp was 3 μg/g. The specific activity of PG preparations from apple mesocarp or exocarp tissues was approximately 3 times less than that found in tomatoes. The pronounced qualitative and quantitative differences between the protein composition of apples and tomatoes became more apparent when protein prepara-

Table I. *Protein content and PG activity of ripe McIntosh apple and tomato fruits*

Plant	Protein	Activity	
	mg g^{-1} fresh wt	*nmol reducing groups min^{-1} g^{-1} fresh wt*	*nkat/mg protein*
Tomato exocarp	0.190	339.3	29.76
Apple			
Exocarp	0.003	3.62	20.12
Mesocarp	0.001	1.06	17.66

tions of both fruits were subjected to denaturing gel electrophoresis (Fig. 2A). The composition of the cell wall-bound protein fraction from apple fruit tissue also differed from that found in tomato fruits. The major protein bands on denaturing polyacrylamide gels from tomato fruit tissue (Fig. 2A, lane 3) were located at approximate M_rs of 45,000, 40,000, 37,000, 32,000, and 22,000. These bands were not readily recognized in the apple cell wall protein preparation (Fig. 2A, lane 2). Weak bands at M_r 45,000 and 40,000 were present in the apple protein preparations, which corresponded to the protein bands in tomato that were identified as endo-PG by the antibody cross-reaction.

When an identically processed polyacrylamide gel was electroblotted to a nitrocellulose membrane and probed with a polyclonal antibody against the tomato endo-PG, the position of the major protein bands from the apple cell wall protein preparation (Fig. 2B, lane 2), recognized by the antibody as a closely spaced doublet, corresponded to the endo-PG from the tomato fruit tissue (Fig. 2B, lane 3). PG in tomato has the PG2A, PG2B composition (Ali and Brady, 1982) that is caused by the different glycosylation pattern of the PG2 subunit (DellaPenna and Bennett, 1988). In this respect, the PG in apple closely resembles that in tomato. The apple cell wall protein preparation also contained a protein band with M_r around 54,000 that cross-reacted with the anti-tomato PG antibody. Such a cross-reacting protein band was also observed in in vitro translation experiments using poly(A)$^+$ RNA isolated from ripe tomato fruits and was identified as an unprocessed form of PG (DellaPenna et al., 1986).

The PG from the McIntosh apples was partially purified by ammonium sulfate fractionation and by gel filtration on a Sephadex G-100 column. Gel filtration resulted in the resolution of a single protein peak that eluted between fractions 35 and 55 (Fig. 3A). This protein peak showed the presence of two enzyme activity peaks (Fig. 3A). The major enzyme activity peak was located in fractions 40 to 44, and the minor activity peak was visible as a pronounced shoulder in fractions 46 to 48. This separation profile was obtained in repeated experiments. PG in both activity peaks was recognized by the antibody preparation (Fig. 3B).

When a commercial pectin preparation was subjected to hydrolysis by an aliquot of fraction 42 of the gel-filtration column that contained the highest PG activity, and the changes in viscosity of the incubation solution were monitored, approximately 70% loss of viscosity was observed during the first 2 h of the reaction (Fig. 4). When the same experiment was performed with the highest activity-containing fraction of the second activity peak (fraction 48), a similar, although less pronounced, decrease in the viscosity of the

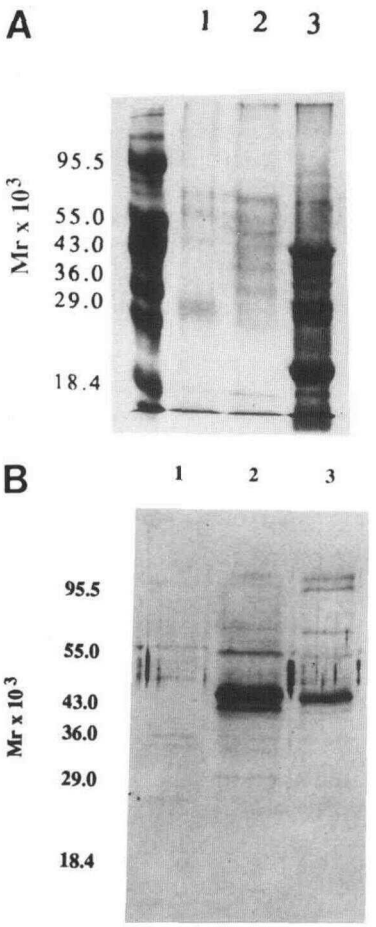

Figure 2. A, SDS-PAGE of apple and tomato PG preparations made from ripe fruit. Lane 1, McIntosh apple exocarp soluble protein (5 μg/well); lane 2, McIntosh apple cell wall extractable protein (5 μg/well); lane 3, tomato exocarp cell wall extractable protein (2 μg/well). The gel was stained with Coomassie brilliant blue. B, Immunoblot of McIntosh apple and tomato protein preparations. An SDS-PAGE identical to that shown in A was electroblotted onto a nitrocellulose membrane and probed with an anti-tomato PG antibody preparation followed by a goat anti-rabbit immunoglobulin G alkaline phosphatase conjugate. Lane 1, McIntosh apple exocarp soluble protein (10 μg); lane 2, McIntosh apple exocarp cell wall extractable protein (10 μg); lane 3, tomato exocarp cell wall extractable protein (1 μg).

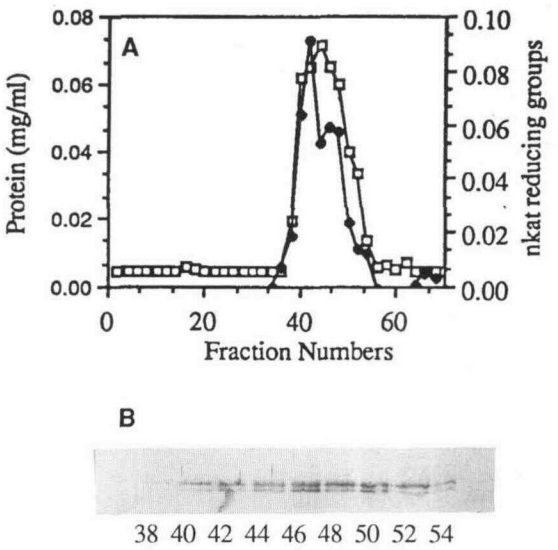

Figure 3. Gel filtration of a PG preparation on a Sephadex G-100 column (A). □, Protein; ◆, PG activity. Enzyme activity-containing fractions were immunoblotted and probed with the anti-tomato PG antibody (B).

incubation mixture was observed (data not shown). When the changes in viscosity obtained with the second, smaller enzyme activity peak were calculated on the basis of protein content in the enzymic reaction mixture (data not shown), the changes in viscosity caused by the enzyme from both activity peaks were identical. These data show that both peaks contained PG.

During the viscosity measurements, samples of the reaction mixture were taken in intervals for determination of the reducing end groups produced by the enzymic reaction and for thin-layer chromatographic analysis. There was little change in the concentration of the reducing groups during the first 2 h of the enzymic hydrolysis of pectin, when most of the viscosity changes took place (Fig. 4). The time course of the reaction producing the reducing end groups progressed slowly and steadily during the hydrolysis of the pectin solution. TLC of the samples taken at 5-min to 12-h intervals up to 48 h showed the absence of monomeric galacturonic acid in the reaction mixtures (data not shown). The results of these experiments are in agreement with the action of an endo-acting PG that is present during the ripening of McIntosh apples.

Results of experiments using a full-length PG cDNA from tomatoes that encodes the expression of an endo enzyme with an M_r of 45,000 supported the presence of an endo-PG in apples. When a poly(A)$^+$ RNA preparation from ripe apples was electrophoresed in a 1% agarose gel, blotted onto a GeneScreen Plus membrane, and probed with pPG1.9, which contains the full-length tomato PG cDNA, an RNA band in the 1.9-kb region showed hybridization (Fig. 5).

Genomic DNA blot hybridization experiments further supported the presence of an endo-PG in apples. When total genomic DNA preparations from remotely related apple cultivars were digested with *Eco*RI, *Hin*dIII, *Eco*RV, *Bgl*II, *Bam*HI, *Xba*I, and *Dra*I restriction enzymes, electrophoresed in agarose, transferred to a GeneScreen Plus membrane, and probed with the full-length tomato PG cDNA probe, hybridizing DNA restriction fragments from the three apple cultivars Robusta 5 (Fig. 6, lanes 1), Rome Beauty (Fig. 6, lanes 2), and White Angel (Fig. 6, lanes 3) were observed, indicating polymorphism of the PG gene in the genus *Malus*.

DISCUSSION

A characteristic process during the ripening of fruits is the softening. Softening is the result of structural changes in the cell wall caused by the activity of hydrolases (Huber, 1983). Although the activity of a number of hydrolytic enzymes has been shown to affect the structure of the cell wall (for a summary, see Huber, 1983), the activity of endo-PG seems to cause the most prominent changes (Bird et al., 1988). Endo-PG has been shown to be present during the ripening of tomatoes (Brady et al., 1982; Grierson, 1985), papayas (Lazan et al., 1989), pears (Pressey and Avants, 1976), and peaches (Lee et al., 1990). Apples, by contrast, were claimed not to contain any endo-PG activity during the ripening process (Pilnik and Voragen, 1970) and were suggested to soften by the activity of an exo enzyme (Bartley, 1978). No firm evidence has been presented in the past for the absence of an endo-PG in apples. However, its absence is widely accepted among investigators (see Kertesz, 1951; Pilnik and Voragen, 1970; Bartley, 1978; Abeles and Biles, 1991).

Reports of the absence of endo-PG in apples were most probably caused by the low amounts of protein and, hence, PG activity in this fruit tissue. As shown in Table I, the protein content of apple tissues is approximately 50- to 100-fold lower than that of tomato fruits and so is the corresponding PG activity on a gram fresh weight basis. However, the specific activity of the PG in apples is similar to that found in tomatoes.

Contrary to a recent report (Abeles and Biles, 1991), PG activity is clearly expressed during development and maturation of apple fruits. Figure 1 shows that although 6-week-old apple fruits (sample date July 5) do not contain detectable activity of the enzyme, PG activity is detected in 11-week-old fruits (sample date August 17), and this activity increases throughout maturation and ripening. Immunoblots of the

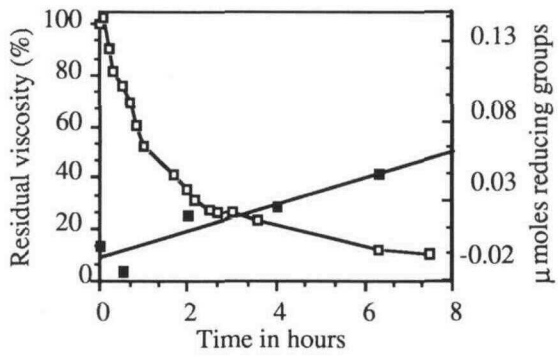

Figure 4. Changes in viscosity (□) and reducing end groups (■) of a pectin solution during incubation with fraction 42, the highest PG activity-containing fraction from the gel filtration column.

samples reveal two protein bands recognized by an antibody preparation that was developed against the tomato enzyme, and the M_rs of these protein bands corresponded to the endo-PG bands (Fig. 2A) that was described in tomatoes (Ali and Brady, 1982; DellaPenna et al., 1990).

We have partially purified the PG from ripe McIntosh apples and investigated its mode of action. Gel filtration indicated the possible presence of a higher and a lower M_r PG in McIntosh apples (Fig. 3A); the two activity peaks had estimated M_rs of 61,000 and 56,000, respectively. Immunoblotting of these peaks, however, showed M_rs of 46,000 and 44,000 that were similar to the enzyme reported from tomato fruits (Grierson, 1985). The M_rs of the PG estimated from the gel-filtration column are approximately that reported by Bartley (1978) as the M_r of "exo-PG," 58,000. The discrepancy in the M_r of the PG determined by gel filtration and immunoblotting is most likely caused by adsorption of the enzyme to the polyglucan matrix of the gel-filtration column, leading to erroneous estimation of the enzyme's M_r in Bartley's experiments. Immunoblotting of the enzyme activity-containing fractions showed that enzyme activity in both peak areas was caused by the presence of the same immunoreactive protein. Therefore, these data do not support the presence of an exo-PG in either of the two enzyme activity peaks.

The report on an exo-PG in apples was based mainly on data derived from molecular mass estimation of the enzyme and viscosity measurements of pectin solutions in the presence of PG preparations (Bartley, 1978). Therefore, we carried out viscosity measurements with the partially purified PG preparation from apple fruits. Viscosity measurements with the highest activity fractions from both peaks showed curves with identical shapes and time courses (Fig. 4, data are shown with the higher enzyme activity peak only). The rapid decrease in viscosity of the enzymic reaction mixture during the first 2 h of the incubation (70%), a relatively slow increase of the reducing groups, and the absence of monomeric galacturonic acid during the entire time course of the reaction identified the PG in McIntosh apples as an endo-acting enzyme. Additional evidence for the presence of an endo-PG in apples was provided by the data from experiments that indicated identity of PG genes in apple with that in tomato fruits. As shown in Figure 5, mRNAs isolated from ripe apples hybridized with a full-length tomato PG cDNA clone (DellaPenna et al., 1986) in northern blotting experiments. In Southern hybridization experiments (Fig. 6), PG DNA could be detected in such remotely related apple cultivars as Robusta 5, Rome Beauty, and White Angel and showed polymorphism among the cultivars.

Our data deriving from the above-discussed biochemical, immunological, and molecular experiments clearly show the presence of an endo-PG in apples, the activity of which is expressed during maturation and ripening. Our report is, therefore, contrary to previous reports that claimed the absence of this enzyme in apples (Kertesz, 1951; Pilnik and Voragen, 1970; Bartley, 1978; Abeles and Biles, 1991).

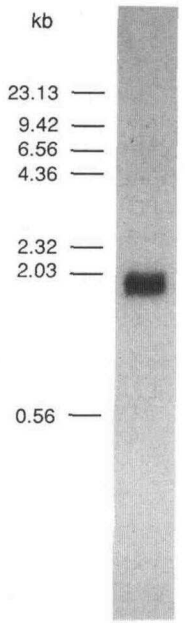

Figure 5. Northern analysis of a McIntosh apple RNA preparation. RNA was isolated from ripe fruit as described in "Materials and Methods." Poly(A)$^+$ RNA (10 μg) was electrophoresed in a 1% formaldehyde denaturing agarose gel and blotted onto a GeneScreen Plus membrane. The membrane was hybridized with an insert of pPG1.9 full-length tomato PG cDNA.

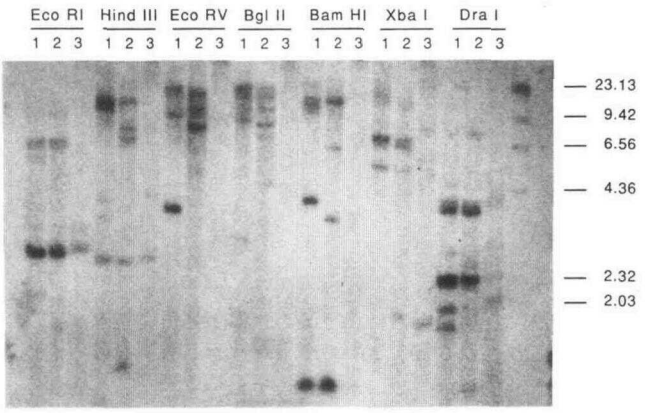

Figure 6. Southern blot hybridization of apple genomic DNA. Apple genomic DNA was prepared as described in "Materials and Methods." The DNA preparations (15 μg) of Robusta 5 (lanes 1), Rome Beauty (lanes 2), and White Angel (lanes 3) apple cultivars were digested with *Eco*RI, *Hin*dIII, *Eco*RV, *Bgl*II, *Bam*HI, *Xba*I, and *Dra*I restriction enzymes, separated in a 1% agarose gel, and blotted onto a GeneScreen Plus membrane. Hybridization of the membrane was carried out as described in "Materials and Methods."

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