Reevaluation of the changes in polygalacturonases in tomatoes during ripening

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Abstract. A procedure was developed for the differential extraction of polygalacturonases (PG) I and II from tomatoes (Lycopersicon esculentum Mill.). Extraction of pericarp tissue from ripe fruit at conventional conditions of 1.0 M NaCl and pH 6.0 yielded nearly equal amounts of the two enzymes. However, most of the PG activity could be extracted also with water at pH 1.6, and the water extract contained only PG II. Subsequent extraction of the pellet with 1.0 M NaCl at pH 6.0 and 10.0 yielded some PG I and high levels of PG converter, the protein in tomatoes that reacts with PG II to form PG I. Application of this procedure to tomatoes at different stages of ripening showed that PG II appeared as ripening began and then increased during ripening. Much lower levels of PG I than of PG II were extracted at all stages of ripeness. The PG converter was present in unripe fruit and increased during ripening. The results demonstrate that PG I is formed when PG II and PG converter are solubilized simultaneously and that PG II is the only endogenous PG in tomatoes.

Key words: Cell wall breakdown - Fruit ripening - Lycopersicon - Polygalacturonase.

Introduction

Ripening of tomatoes is accompanied by the appearance and rapid increase in polygalacturonase (PG) (Hobson 1964; Tucker et al. 1980; Pressey 1986b). Extracts of ripe tomatoes contain two major forms of the enzyme, PG I and PG II (Pressey and Avants 1973). Polygalacturonase I has a much higher molecular weight and is more heat stable than PG II. There has been considerable effort to assign specific roles to the two PGs in the ripening process. Crookes and Grierson (1983) found only PG I in extracts of fruit at the early stages of ripening. Polygalacturonase I increased with ripening and then leveled off when PG II appeared; on further ripening PG II increased to account for 73% of the total PG activity. Crookes and Grierson suggested that PG I was responsible for initiating cell-wall disruption by attacking the middle lamella and that the later hydrolysis of the primary wall was catalyzed by PG II. Their hypothesis appeared to be substantiated by the presence of only PG I in never-ripe fruit, a mutant that softens slowly. Brady et al. (1983) also found that PG I was the first enzyme to be detected in ripening tomatoes but PG II was the major form in ripe fruit. They reported that slowly softening lines of tomatoes accumulated PG I but not PG II, and suggested that PG I may have a particular role in the ripening process.

Except for the greater than twofold difference in molecular size and a large difference in heat stability, PG I and PG II possess remarkably similar properties. They are optimally active at pH 4.0–4.5, their activities shift to the acid side as the substrate size decreases, and both are typical endoenzymes (Pressey and Avants 1973). They possess similar isoelectric points, respond similarly to cations and inhibitors (Ali and Brady 1982) and both are glycoproteins (Moshrefi and Luh 1983). They are equally effective in degrading isolated cell walls from tomatoes (Pressey and Avants 1982). Antibodies raised against PG II reacted also with PG I (Ali and Brady 1982), indicating that portions of the molecules are identical. This is supported by the observations that denaturation of the enzymes with sodium dodecyl sulfate yields similar polypeptides (Tucker et al. 1980; Ali and Brady 1982). The relationship between the two enzymes...
was recently explained in terms of a heat-stable protein (PG converter) that reacts with PG II to form PG I (Pressey 1984a). The PG converter is present in both unripe and ripe tomatoes. The question thus arises whether PG I exists in ripe tomatoes or if it is formed from PG II and PG converter during extraction (Pressey 1986a). In the present study, I provide further evidence that PG I is a product of tissue extraction.

Material and methods

Plant material. Tomatoes (Lycopersicon esculentum Mill.) were grown in a greenhouse under natural light and at day and night temperatures of approx. 25°C and 18°C, respectively. Most of the studies were conducted with the cultivar “Hygro Super X” (Henry Fields Seeds and Nursery, Shenandoah, la., USA), but “Sweet 100”, “Tropic”, “Golden Boy” (Park Seed Co., Greenwood, S.C., USA), “Cornell 111” (Dr. M.A. Mutchler, Cornell University, Ithaca, N.Y., USA) and the mutants nor and rin (Dr. K.C. Gross, U.S. Department of Agriculture, Beltsville, Md.) also were analyzed. Freshly harvested fruit were separated according to the stage of ripeness and were analyzed immediately.

Extraction of PGs and PG converter. Pericarp tissue (50 g) from each of six fruit was homogenized in 100 ml of cold water. The homogenate was adjusted to pH 3.0 with 0.1 N HCl, stirred 10 min and centrifuged at 27000 g for 10 min. The pellet was suspended in 75 ml of cold water (pH 3.0), stirred 10 min and centrifuged. The washed cell-wall fragments were then extracted at various conditions of NaCl concentration and pH for 10 min and centrifuged at 27000 g for 10 min. Some of the pellets were reextracted at different conditions. All of these steps were conducted near 0°C.

Assays for PG and PG converter. The total PG activity in the extracts was measured by adding 0.1 ml of the extract to 0.2 ml of 0.1 M sodium acetate (pH 4.5) and 0.2 ml of 0.15 M NaCl. Blanks were prepared by heating duplicate samples 5 min at 100°C. The reactions were started by adding 0.5 ml of 1% polygalacturonic acid (Pressey and Avants 1973), adjusted to pH 4.5. After 30 min at 37°C, the solutions were analyzed for reducing groups by the arsenomolybdate method (Nelson 1944). Polygalacturonase I in the extracts was measured by preparing reaction mixtures as described above and heating 5 min at 65°C before the addition of substrate (Tucker et al. 1980). The difference between the total PG activity and PG I represented PG II. A unit of PG activity is defined as the amount that released 1 μmol of reducing groups per 30 min.

The PG converter was assayed by measuring the conversion of PG II to PG I. The reaction mixture consisted of 0.1 ml of extract, 0.2 ml of sodium acetate (pH 4.5) and 0.2 ml of 0.15 M NaCl containing 4 units of purified PG II (Pressey 1984a). After 10 min at 37°C, the samples were heated 5 min at 65°C, cooled to 37°C, 0.5 ml of substrate was added to each sample and the PG activity was assayed as above. A unit of PG converter was defined as that amount that converted one unit of PG II to PG I.

Results

As reported earlier, the extractability of PG from tomatoes was highly dependent on the pH and salt concentration of the extraction medium (Pressey 1986a). Polygalacturonase was not extracted by water from cell-wall fragments between pH 2.0 and 3.5. Extractability of PG by water increased as the pH was raised to a maximum near pH 6.5, and these extracts contained both PG I and PG II. Polygalacturonase was also extracted from the cell-wall fragments as the pH was lowered below 2.0 but these extracts appeared to contain only PG II. The differential extraction of the PGs at acid and neutral conditions indicated that this would be a useful approach for studying the presence and changes of PG I and PG II during ripening.

Preliminary studies were conducted to determine the stabilities of PG I and PG II at low pH. The two enzymes were purified from ripe tomatoes according to the procedures described in Pressey and Avants (1982). The enzyme solutions were diluted to approx. 20 units/ml, adjusted to pH 1.6 by addition of 0.1 N HCl, and stored at 4°C. Aliquots were withdrawn over a 6-h period, adjusted to pH 4.5, and assayed for polygalacturonase (Table 1). Polygalacturonase I also was assayed after heating at 65°C for 5 min to measure conversion to PG II. Both enzymes lost activity very slowly at pH 1.6, and PG I did not appear to be converted to PG II. The latter was confirmed by analyzing the PG I solution after 6 h at pH 1.6 for PG isoenzymes by high-pressure liquid chromatography (Pressey 1984b). The only detectable enzyme in the solution was PG I.

The original extraction procedure (Pressey 1986a) was modified to minimize the small losses of PG activity at low pH. This was accomplished by keeping the extracts at near 0°C, reducing the extraction time to 10 min, and decreasing the centrifugation time to 10 min by increasing the speed to 27000 g. The supernatant solutions obtained by centrifugation were immediately adjusted to a

<table>
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<th>Time (h)</th>
<th>PG I (unheated)</th>
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