Cellulase Activity and Fruit Softening in Avocado

EDNA PESIS, YORAM FUCHS, AND GIORA ZAUBERMAN

Division of Fruit and Vegetable Storage, Institute for Technology and Storage of Agricultural Products, Agricultural Research Organization, The Volcani Center, P.O. Box 6, Bet Dagan, Israel

Received for publication May 24, 1977 and in revised form October 18, 1977

ABSTRACT

Cellulase activity in detached avocado (Persea americana Mill.) fruits was found to be directly correlated with ripening processes such as climacteric rise of respiration, ethylene evolution, and softening. This activity in the pericarp could be induced by ethylene treatment, and the more mature the fruit—the faster and the greater was the response. Only a very low cellulase activity could be detected in hard avocado fruit right after harvest. Cellulase activity was highest at the distal end of the fruit, lower in the midsection, and lowest at the proximal end. The enzyme is heat-labile and appeared to have activity of endocellulase nature mainly. Electron micrographs of cell walls from hard and soft fruits are presented.

The most obvious feature of avocado fruit ripening is softening, and the most common physiological parameters for determining avocado ripening are ethylene evolution and respiration rate (4). It is generally believed that softening of fruits during ripening is related to alteration in the pectic substances through action of pectic enzymes (4). A clear correlation between the activities of polygalacturonase and pectin methylesterase and fruit softening in avocado has been shown (3, 23). Also, differences in polygalacturonase activity (17) and in ethylene production and respiration (21) between the different parts of avocado fruit were reported. It has been suggested that cellulase, in addition to pectic enzymes, might contribute to softening of tomato fruits (7, 13, 15) and during development and of dates (12). Cellulose was reported to be the main constituent of young avocado fruit cell walls (19). Cellulase activity in freeze-injured avocado fruits has been reported (10). The ultrastructure of plant cell walls has been described in detail (2, 14), and some ultrastructural aspects of avocado fruit have been reported (19).

It was of interest to study what might be the involvement of cellulase during normal and ethylene-induced ripening processes in avocado. Therefore, changes in cellulase activity were investigated in fruits which were harvested at various stages of maturity and treated with ethylene. Some characteristics of the partially purified enzyme and some ultrastructural changes in the cell walls were studied.

MATERIALS AND METHODS

Plant Material. Avocado (Persea americana Mill.) fruits of the 'Fuerte' cultivar were harvested periodically, starting in June with very young fruit (62 g and 1.6% oil content), and ending in December with completely mature fruit (313 g and 14.9% oil content). These fruits were stored at 20 C. Starting on the day of harvest, ethylene was applied for 48 hr in a flow system, at a concentration of 50 μl/l; pure air was supplied to the controls.

Acetone powder was prepared from fruit pulp: 1 part of pulp and 8 parts (w/v) of acetone at −20 C were homogenized in a blender. After vacuum filtration the powder was blended a few more times with cold acetone until a bright colorless powder was obtained. The powder was dried at room temperature and then milled in a coffee mill and stored at −20 C until extraction.

Tissue for electron microscopy was fixed from mature fruits (17.4% oil content, 11.2 kg firmness) on the day of harvest and after 11 days of storage in the dark at 14 C. These fruits were also used for the studies summarized in Figure 2.

Electron Microscopy. Tissue (1 x 1 mm) from the "pale green" zone of the pericarp was fixed at room temperature for 2 hr with 4% glutaraldehyde and 0.1 M phosphate buffer (pH 7.5). The tissue was rinsed in buffer and postfixed in 2% OsO4 in the same buffer for 1 hr at room temperature. It was dehydrated in an alcohol series followed by propylene oxide and embedded in Epon 812. Silver-gray sections were cut, mounted on copper grids, stained in uranyl acetate-lead citrate, and viewed in a Jeol electron microscope (JEM-T7).

Enzyme Extraction and Partial Purification. Acetone powder (400 mg) was extracted with 10 ml of 0.02 M phosphate buffer at pH 6.6 for 1 hr at 1 C. Also, extractions were tested with 1 M NaCl added to the extracting buffer. The slurry was centrifuged for 20 min at 30,000 g at 1 C. The supernatant was decanted and then filtered through a Millipore filter (1.2 μ). One ml of the filtrate was applied on a Sephadex G-25 column (1.7 x 18 cm) and eluted with 0.02 M phosphate buffer at pH 6.6. Fractions of 5 ml were collected and assayed for cellulase activity, and the optical density at 280 nm was measured for protein content determination, using a BSA calibration curve (Fig. 1). The fractions containing the main cellulase activity were combined to give the "enzyme solution."

Cellulase Assay. Enzyme solution (5 ml) and 10 ml of substrate (1% CMC, 2 BDH), dissolved in 0.1 M acetate buffer at pH 5.5 containing 0.1% synthymocetin and 0.1% cycloheximide, were incubated at 30 C in an Ostwald viscometer head (Volac No. 150), and readings were recorded at 10-min intervals for 60 min. The optimal pH was found to be between 4.5 and 6.0. A unit of cellulase activity was defined as the amount of solution which causes a 1% loss in relative viscosity/hr of the reaction mixture. A reaction mixture containing 5 ml of boiled enzyme solution was used as a blank.

Reducing Groups. In some of the experiments Sumner's reagent (20) was used to measure changes in reducing groups as an indication of cellulase activity. For this determination, 5 ml of enzyme solution was incubated with 10 ml of the substrate (CMC as above) at 30 C, and 1 ml of this reaction mixture was boiled 5 min with 2 ml of Sumner's reagent; the reduction product was determined colorimetrically at 550 nm.

Fruit Firmness. Firmness was determined, without removing the epidermis, by a Chatillon pressure tester, using the conical tip, in which the number of kg force to penetrate the pulp is directly correlated to firmness. At about 3.5 kg the beginning of softening could be felt by hand.

1 Contribution from the Agricultural Research Organization, The Volcani Center, Bet Dagan, Israel. 2 Abbreviation: CMC, carboxymethyl cellulose.
Ethylene and CO₂ were determined by gas chromatography (9), and oil content was determined using a refractive index method (11). Data represent at least six experiments which were carried out during two seasons; either averages or representative curves are presented.

RESULTS

Very low cellulase activity was detected on the day of harvest in pericarp from avocado fruits which were harvested at various stages of development. Activity was noticeable only when the fruit began to go through its climacteric rise in respiration and to produce ethylene, but before softening was evident (Fig. 2). No significant differences in final cellulase activity levels were observed between young immature and mature fruits. However, cellulase activity and ripening, in general, became evident more rapidly after harvest in mature fruit than in young fruit. Maximal cellulase activity was attained when the fruit was completely soft (Fig. 2). Cellulase activity during ripening was highest in the distal end, lower in the midsection, and lowest in the proximal end (Fig. 3), while the firmness of these different parts of the fruit was inversely correlated to their cellulase activity. In completely soft fruit no significant differences in cellulase activity or firmness, could be observed among the different parts of the fruit.

In Figure 4 the differences in the structure of cell walls from hard and soft fruits are demonstrated. In the hard fruit (Fig. 4, A and B) the middle lamella is obvious and the fibrils are packed tightly in order on both sides of the middle lamella. In soft fruit (Fig. 4, C and D) no middle lamella can be observed, many of the fibrils are missing, and many diagonal fibrils can be seen.

Ethylene treatment had no effect on cellulase activity (measured 48 hr after harvest) in very young fruits harvested in June or July (Fig. 5); later in the season, the more mature the fruit—the higher was the cellulase activity and the more it responded to ethylene. There was a lag in the increase in the reducing power of the enzymic reaction mixture as compared with the decrease in viscosity (Fig. 6). Addition of 1 M NaCl to the extracting buffer did not affect the cellulase activity of the extract. Incubating the enzyme solution for 0.5 or 2 hr at 30°C before the assay did not affect its activity; incubation for the same length of time at 40, 50, and 60°C inactivated the enzyme, and the higher the temperature and the longer the time—the greater was the inactivation.

DISCUSSION

Since cellulase activity in the avocado fruit increased during ripening (Fig. 2), it is possible that the enzyme has a role in ripening processes, perhaps in conjunction with polygalacturonase (23). It has been suggested (7) that cellulase may act in tomato fruit in conjunction with pectic enzymes to cause softening. The fact that the increase in cellulase activity matches the changes in the ripening, such as increase in respiration rate and ethylene evolution (Fig. 2), is additional supporting evidence for the involvement of cellulase in avocado softening.

In our studies with ‘Fuerte’ fruits, softening and cellulase activity always occurred earliest at the distal end of the fruit (Fig. 3), as has been previously reported for polygalacturonase activity in this variety (17). In their studies with the ‘Hass’ variety, Tingwa and Young (21) reported that ethylene production and softening of the fruit were always detected earlier in the stem end than in other parts of the fruit. It seems that there are differences in the sequence of ripening, within the fruit, between the two varieties.

One would expect that as a result of the action of pectic and cellulytic enzymes, pectins and cellulose in the fruit would be hydrolyzed; indeed, it can be observed that the middle lamella and the orderly tight fibrils which are evident in hard fruit (Fig. 4, A and B) are dissolved and sparse, respectively, in soft fruit.

Copyright © 1978 American Society of Plant Biologists. All rights reserved.
FIG. 4. Electron micrographs of avocado fruit cell walls prepared from: A: hard (firm) fruit (× 29,400); B: hard (firm) fruit (× 29,400); C: soft fruit (× 50,400); D: soft fruit (× 70,000). ml: middle lamella; cw: cell wall; cy: cytoplasm; v: vacuole.
Cellulase and softening in avocado

Cellulose in vivo is not a single enzyme but a group of isoenzymes, and the activity of these isoenzymes is controlled by ethylene. The ethylene induces cellulase activity in avocado fruit, and the increase in cellulase activity is correlated with the softening of the fruit. The increase in cellulase activity is not only due to the induction of new enzyme but also to an increase in the activity of existing enzyme.

The activity of cellulase in avocado fruit is strongly influenced by ethylene. The ethylene treatment increases the cellulase activity, and the increase in cellulase activity is accompanied by the softening of the fruit. The increase in cellulase activity is not only due to the induction of new enzyme but also to an increase in the activity of existing enzyme.

Acknowledgment—We thank E. Zamski for his help with the electron microscopy studies.

LITERATURE CITED

2. ALBERSHEIM P 1975 The wall of growing plant cells. Sci Am 232: 81–95
15. PHARR DM, DB DICKINSON 1973 Partial characterization of Cx cellulase and cellobiase from ripening tomato fruits. Plant Physiol 51: 577–583