Communication

Cell Turgor Changes Associated with Ripening in Tomato Pericarp Tissue

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ABSTRACT

The pressure microprobe was used to determine whether the turgor pressure in tomato (Lycopersicon esculentum Mill., variety "Castelmart") pericarp cells changed during fruit ripening. The turgor pressure of cells located 200 to 500 micrometers below the fruit epidermis was uniform within the same tissue (typically ± 0.02 megapascals), and the highest turgors observed (<0.2 megapascals) were much less than expected, based on tissue osmotic potential (~0.8 to ~0.7 megapascals). These low turgor values may indicate the presence of apoplastic solutes. In both intact fruit and cultured discs of pericarp tissue, a small increase in turgor preceded the onset of ripening, and a decrease in turgor occurred during ripening. Differences in the turgor of individual intact fruit occurred 2 to 4 days before parallel differences in their ripening behavior were apparent, indicating that changes in turgor may reflect physiological changes at the cell level that precede expression of ripening at the tissue level.

Postharvest decreases in fruit firmness (fruit softening) are an important component of the increase in palatability that accompanies fruit ripening. If softening is not effectively controlled in the postharvest environment, however, fruit susceptibility to mechanical damage and pathogen attack is greatly increased (16). Consequently, there have been several investigations into the cellular processes that regulate softening and into postharvest handling procedures that can slow or stop the process (1, 8, 18). Most of the work concerning the biochemical basis of fruit softening has emphasized the metabolism of the cell wall/middle lamella complex. It is reasonable to presume that softening is a physical consequence of cell wall breakdown, since cells in ripening fruits are more readily crushed and/or can be moved past each other more easily than cells in nonripening fruit. A straightforward relation between fruit softening and the breakdown of fruit cell wall pectins and, to a lesser extent, hemicelluloses has been developed in the literature (9). This remains the dominant model explaining softening, although recent work (e.g. 6) has indicated that softening requires more than the digestion of pectin. Application of calcium to unripe fruit has been shown to substantially reduce fruit softening in storage and slow the rate of ripening (14). This too has been interpreted in terms of cell wall integrity—the idea being that Ca\textsuperscript{2+} has induced a stiffening of cell wall pectin gels (7).

Fruit firmness is quantified by measuring the force required to physically compress or penetrate external tissues. This force may change during softening as a result of reduced integrity in cell wall components but could also be a consequence of changes in the hydrostatic pressure (turgor) within fruit cells. Since cell turgor requires maintenance of membrane integrity, the impact of Ca\textsuperscript{2+} on firmness may also be understood as a turgor-mediated consequence of the known membrane stabilizing properties of Ca\textsuperscript{2+} (3, 13). To our knowledge, there are no previous reports of changes in cell turgor associated with the ripening process in any fruit. Steudle and Wieneke (17) reported that elastic and hydraulic properties of cells in apple fruit changed during fruit development, but in their system turgor was artificially modified by manipulating the osmotic potential of the bathing medium. Using the pressure microprobe technique (11), this communication reports direct measurements of changes in turgor in the pericarp cells of ripening intact tomato fruit, and in a recently described in vitro ripening system, the tomato pericarp disc (2).

MATERIALS AND METHODS

Plant Material and Experimental Design

Field-grown tomatoes (Lycopersicon esculentum Mill.) var. "Castelmart" were harvested from university experimental plots at Davis, CA, at the mature-green 3 to 4 stage of maturity, and, in most cases, were stored at 10\textdegree C for 1 to 2 d before use. Disc preparation has been described previously (2). Briefly, cylinders of pericarp tissue (10-mm diameter, 5–7 mm thick) were excised from surface-sterilized fruits (about 10 discs/fruit) and placed epidermis-side-down in multi-well plates (Falcon 3047). Both discs and intact fruits were incubated (ripened) at 20\textdegree C in boxes flushed with 3 volumes of water saturated air per hour to prevent desiccation. Preliminary experiments were performed on freshly cut discs from mature-green and red-ripe fruit, and two experiments were performed to follow turgor and color changes during ripening of discs and whole fruits. In experiment 1, fruits were harvested on September 19, 1990, and a total of six intact fruits and an initial population of 240 individual discs were ripened in culture (2). Color (see below) was measured nondestructively on all discs (one measurement per disc) and fruits (three random measurements per fruit) on a daily basis. Any discs showing signs of microbial contamination or tissue damage were discarded. Periodic measurements of turgor were made on each fruit, and on four discs with skin color close to that of the disc population mean for the day of measurement.

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Turgor measurements were obtained from two cells in the center of each disc and three cells at random positions on each fruit at each time of sampling. After turgor measurement, discs were discarded and fruits were returned to the ripening conditions. In experiment 2, fruits were harvested on October 4, 1990, and a total of five intact fruits and an initial population of 120 individual discs were ripened. Disc and fruit color were measured nondestructively as in experiment 1, but fruit color was sampled from the same three positions on each fruit for each time of measurement. The same protocol as in experiment 1 was used for turgor measurement, but after measurement, discs were sealed in plastic vials and frozen for later determination of osmotic potential.

Measurement of Fruit Color and Water Relations

Fruit color was measured directly on the fruit surface, and disc color through the clear plastic base of the multi-well plate, with a reflectance colorimeter (Minolta CR-200). Colors reported are for the a* component of the L*a*b* uniform color space (CIELAB), which measures hue on a green (−) to red (+) axis (10). Disc color was corrected for the effect of the plastic (2).

The pressure microprobe (11) was used to measure the turgor of cells located 200 to 500 μm below the epidermis in both whole fruit and pericarp discs. Tissue penetration and meniscus behavior were observed at ×200 through a vertically illuminated microscope with a long distance objective (15). The methodology used to measure the turgor of subepidermal cells was essentially the same as that described by Cosgrove and Cleland (5), in which the epidermal and first few subepidermal cells were sacrificed to establish an observable meniscus external to the tissue. As was found by Cosgrove and Cleland (5), after meniscus establishment the measured turgor of sequentially penetrated subepidermal cells was quite uniform (typically ± 0.02 MPa). Difficulties of tip breakage were experienced, such as those described by Steudle and Wiencek (17), in the initial attempts to penetrate the fruit epidermis. These were solved by producing very short microcapillary tips (pulled with a Koph Mod. 750 micropipette puller) that were widened and sharpened with a modified jet-stream microbeveler (12). For turgor measurements, individual pericarp discs were removed from culture and placed, epidermal-side-up, in a plastic block with a cylindrical cavity of about the same depth and diameter as the disc. In this way the tissue could be easily positioned during turgor measurements, while minimizing the exposure of disc culture surface. A few drops of water were placed in the cavity to prevent tissue drying during turgor measurements, which were performed under laboratory conditions (diffuse fluorescent light and 25–30°C air temperature) and were completed within 40 min of removing discs from culture. During this period of time, there was no detectable change in the turgor of the subepidermal cells located in the center of the 10-mm-diameter disc. Whole fruit turgor measurements were performed under the same laboratory conditions. Osmotic potentials of sap extracted from frozen-thawed pericarp discs were determined with a vapor pressure osmometer (5500; Wescor, Inc., Logan, UT).

RESULTS AND DISCUSSION

In preliminary experiments, the turgor in discs cut from mature-green fruit was always found to be higher than that in

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**Table 1. Turgor of the Subepidermal Cells in Pericarp Discs Cut from Mature-Green and Red-Ripe Maturity Stage Tomato Fruit**

<table>
<thead>
<tr>
<th>Maturity Stage</th>
<th>Turgor MPa*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mature-green</td>
<td>0.14</td>
</tr>
<tr>
<td>Red-ripe</td>
<td>0.03</td>
</tr>
</tbody>
</table>

* Mean of 17 cells sampled from seven individual fruits. ** LSD (P < 0.01) of 0.02 was obtained from a simple one-way ANOVA.

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Figure 1. Changes in subepidermal cell turgor and in surface color (a*, measuring hue on a green [−] to red [+] axis, [10]) of tomato pericarp discs (experiments 1 and 2) and intact tomato fruit (experiment 1 only) during ripening at 20°C. Each point shows the mean ± 2 se, and errors smaller than the symbols are hidden. The number of measurements (n) in each mean is: experiment 1 disc color n = 200, disc turgor n = 8, fruit color n = 18, fruit turgor n = 8; experiment 2 disc color n = 100 and disc turgor n = 14. Only the initial, minimum, and final values (d 1, 4, and 13, respectively) of disc osmotic potential in experiment 2 (n = 2, pooled se = 0.3 MPa) are indicated at the top.

Figure 2. Changes in subepidermal cell turgor and in surface color of individual intact tomato fruit (experiment 2) ripening independently at 20°C. Each point shows the mean ± 2 se, and errors smaller than the symbols are hidden. The number of measurements in each turgor mean is 2 and in each color mean is 3.
discs cut from red-ripe fruit (Table I). These turgors, however, were substantially less than those expected based on the range of osmotic potentials found in these tissues (−0.6—−0.7 MPa), even when discs had been partly or fully submerged in water for 4 h (data not shown). Under such conditions, it is expected that tissue hydration would be essentially complete and that cell turgor and cell osmotic potentials would be equal and opposite. It is possible that damage to the cell wall during penetration could result in erroneously low measured turgors, but the turgor of sequentially penetrated cells was uniform (as described by Cosgrove and Cleland [5]) and stable immediately after penetration (as described by Shackel et al. [15]) for all reported turgor values. Hence, there was no evidence to support the hypothesis that these low turgors were the result of cell wall damage. One physiological mechanism that could account for low turgors is the presence of solutes in the apoplastic space of this tissue, as suggested for hypocotyl tissue by Cosgrove and Cleland (4). Regardless of the mechanism, however, these data indicate that the turgor of the pericarp cells of mature green tomato fruit is relatively low and is further reduced during ripening.

During ripening in both discs and intact fruits, a decline in turgor occurred, although the rate of ripening (as measured by skin color) and also the absolute level of turgor were different in different experiments (Fig. 1). In all cases, however, the maximum turgor occurred 3 to 4 d after the start of incubation, followed by a decline in turgor as ripening progressed. The osmotic potentials of the discs in experiment 2 exhibited an overall pattern of change that was opposite that of their turgor, namely an initial decrease followed by a subsequent increase (only initial, minimum, and final values are indicated in Fig. 1). The point of minimum osmotic potential, however, did not correspond to the point of maximum turgor, nor did the overall decrease in turgor correspond to an overall increase in osmotic potential. Hence, there was no simple relation between tissue osmotic potential and cell turgor potential during fruit ripening. If apoplastic solutes are present in these tissues, however, then this lack of relation may not be surprising, since a change in partitioning of solutes between sympatric and apoplastic compartments could alter cell turgor without any change occurring in whole tissue osmotic potential. If the initial decrease in whole tissue osmotic potential reflected osmotic changes in the symplasm, then it was large enough to easily account for the initial increase in turgor observed in this study.

In experiment 1, the rate of color change (ripening) in intact fruits roughly corresponded to the average rate of color change in the discs (Fig. 1). However, individual fruits ripened independently, so that on the same date, color variation between fruits was higher than color variation within fruits. Independent fruit ripening was also exhibited in experiment 2, and for the three individual fruits that ripened normally during this experiment, the order of fruit ripening was identical to the order of occurrence in the maximum observed fruit turgor (Fig. 2). Since the maximum turgor in these fruits was exhibited 2 to 4 d before any change was apparent in fruit color, these data suggest that changes in turgor may reflect physiological changes at the cell or cell membrane level that precede the expression of ripening at the tissue level.

Campbell et al. (2) have shown that ripening in pericarp discs (as judged by color change, increased CO₂ evolution and ethylene production, and, especially pertinent to this report, decreased tissue firmness and altered cell wall chemistry) follows the same course as ripening in intact fruit. The data shown in Figures 1 and 2 suggest that decreases in turgor follow skin reddening in much the same way as wall chemical changes and softening (2). It is thus possible that some aspects of tomato softening are the result of turgor loss as well as (or instead of) altered wall integrity. Figure 2 also suggests that physiological changes in cell membranes or symplast/apoplast osmotic relations may precede the turgor loss that is associated with ripening. Further study of these changes may shed additional light on the physiological basis of softening and other important metabolic aspects of the ripening process.

LITERATURE CITED