Induction and Regulation of Ethylene Biosynthesis and Ripening by Pectic Oligomers in Tomato Pericarp Discs

Alan D. Campbell* and John M. Labavitch

Department of Pomology, University of California at Davis, Davis, California 95616

ABSTRACT

The effect of pectic oligomers and 1-aminocyclopropane carboxylic acid on ethylene biosynthesis and color change was studied in ripening tomato pericarp discs excised from mature-green tomato fruit (Lycopersicon esculentum Mill.). Pectic oligomers induced at least four distinct responses when added to pericarp discs: (a) a short-term, transient increase in ethylene biosynthesis; (b) a long-term, persistent increase in climacteric ethylene in discs excised from mature-green fruit; (c) an advance in ripening processes, as indicated by increased reddening of the disc surfaces; and (d) a darkening of the treated endocarp surface. Pectic oligomers appear to affect the ripening of exocarp and endocarp tissues by different mechanisms. In exocarp tissues, the acceleration of reddening by pectic oligomers might simply be a consequence of induced ethylene biosynthesis. In endocarp tissues, the acceleration of reddening appears to be a direct effect of oligomers on ripening processes. We suggest that the rate of ripening of endocarp tissues may be regulated, in part, by the release of pectic oligomers from the cell walls of adjacent exocarp tissues. Exocarp and endocarp tissues of pericarp discs appear to differ in their sensitivity to ethylene at each maturity stage, and to exhibit independent changes in sensitivity to ethylene as ripening progresses. The tissue-specific pattern of reddening in tomato pericarp may result from this differential sensitivity to endogenous ethylene concentrations.

The phenomenon of climacteric ripening clearly encompasses a diverse set of physiological processes. In tomato fruit (Lycopersicon esculentum Mill.) obvious changes in color, texture, flavor, and aroma progress through an anatomically complex fruit in just a few days (2, 13). Color change in tomato occurs in a rather circuitous pattern. In a typical fruit, loss of chlorophyll and synthesis of lycopene begin in the locular or central columnella tissues, emerge externally at the stylar end of the fruit, then spread rapidly across the superficial exocarp layer, before progressing into the underlying endocarp tissues (13, 23). Changes in ethylene biosynthetic activity (4) and PG2 activity (26) have been recently shown to follow a similar circuitous pattern. The regulatory mechanisms which are responsible for this complex spatial pattern of ripening are unknown.

Ethylene plays a critical role in the induction and coordination of ripening processes in climacteric fruit. The rise in ethylene biosynthesis is an early event in tomato ripening (14, 25), and continued ethylene synthesis (11) and action (10) appear necessary to the continuation of ripening processes. The progressive increase in internal ethylene concentration may control the temporal sequence of expression of some ripening-related genes in pericarp tissues (21). However, because of the rapid diffusion of ethylene within the tomato fruit (6), it seems unlikely that local differences in ethylene concentration can be responsible for the spatial sequence of tomato ripening. We have noted that green pericarp discs removed from the ethylene environment of a whole tomato nonetheless reddened in the same temporal, spatial, and tissue-specific patterns seen in intact fruit (7). The tissue-specific sequence of ripening more likely arises from local differences and differential changes in sensitivity to ethylene, and perhaps from responses to other regulatory signals.

Compositional changes in the cell walls of ripening fruit have received much research attention both because of their influence on fruit firmness (17) and because of a possible role in regulation of ripening processes (2). Tomato ripening is characterized by various changes in cell wall composition, such as a decrease in degree of polymerization of pectic and hemicellulosic fractions and an increase in water-soluble uronides and other carbohydrates within the cell wall (2, 13, 15). PG, a cell wall enzyme which cleaves nonesterified linkages in the α-1,4-polygalacturonic acid backbone of cell wall pectins, has been long thought to control tissue softening in tomato fruit (3, 15, 17) and perhaps to initiate or regulate ripening processes by the release of wall-bound enzymes (27) or regulatory cell-wall fragments (2). Much recent evidence contradicts such a simple role for PG in the regulation of softening or ripening (12, 14, 22, 24), but many of these studies do not address the anatomical complexity of the ripening process in tomato. Whether local differences and changes in ethylene sensitivity or in cell wall composition might regulate the progress of local ripening processes can only be investigated at the tissue level.

We have examined the ripening processes in tomato pericarp discs using a novel system for disc handling which allows

1 This research was supported by the Regional Research U.S. Department of Agriculture Project N.E. 87.
2 Current address: Plant Gene Expression Center, ARS-USDA, 800 Buchanan St., Albany, CA 94710, and Department of Plant Biology, University of California at Berkeley, Berkeley, CA 94720.
3 Abbreviations: PG, polygalacturonase; MG, mature green stage two; MG3, mature green stage three; MG4, mature green stage four; G7, mixture of smaller pectic oligomers; G12, mixture of larger pectic oligomers; ACC 1-aminocyclopropane carboxylic acid; DP, degree of polymerization.
application of treatments to individual discs and subsequent monitoring of ethylene biosynthesis, respiration, and color change (7). Pericarp discs excised from mature-green tomato fruit undergo progressive changes which closely model those which occur during ripening in whole fruit. In these discs, ethylene biosynthesis and respiration undergo a climacteric rise, skin color changes from green to red, tissues soften, hydrolytic enzymes increase, cell wall composition changes, and characteristic aromas and flavors develop in a spatial and temporal pattern similar to that observed in intact fruit (7). The outlined experiments were undertaken to determine if exogenous pectic oligomers have an effect on tomato pericarp discs consistent with a role for endogenous oligomers in the regulation of ripening processes in tomato (8).

MATERIALS AND METHODS

Tomato Fruit

Field-grown tomato (Lycopersicon esculentum Mill.) fruit from variety '674' were obtained at the mature-green stage from Florida, through Bianchi and Sons Packing, Merced, CA. Unblemished fruit, 150 to 200 g in mass, were washed, placed in open 490-mL jars, and kept at 20°C. Rates of ethylene biosynthesis by intact fruit were measured daily. Jars were capped and accumulated gases sampled after 30 min. Ethylene was separated by gas chromatography at 80°C with an alumina column and quantified by the integration of the peak from a flame ionization detector. Fruit for disc preparation were selected at three progressive maturity stages: MG2, at which fruit are capable of ripening, but the locules are only partly filled with gel; MG3, at which fruit are capable of ripening and the locules are full, but where no internal color change is visible; and MG4, at which ripening has begun with the appearance of internal color change. Transition from MG2 to MG3 is usually accompanied by a stable two- to threefold increase in ethylene biosynthesis. Transition from MG3 to MG4 is marked by the onset of a steady rise in ethylene biosynthesis (13, 23).

Disc Preparation and Handling

Procedures for preparation, handling, and measurement of pericarp discs are more thoroughly described elsewhere (7). Disc preparation was carried out with sterile technique in a sterile hood using procedures developed by M. Saltveit (11). The selected fruit were briefly sterilized in 1% sodium hypochlorite and rinsed thoroughly with water. Cylinders of pericarp were cut from the equatorial region with a cork borer, then sliced by hand into discs with epidermis included. Discs were approximately 1 cm in diameter, 3 mm in thickness, and 300 mg in weight, with up to 96 discs prepared from a single fruit. Cut discs were briefly rinsed twice with distilled water, drained, and then blotted, cut surface down, on sterile filter paper for about 60 s to remove absorbed water. Blotted discs were placed in individual wells of sterile, 24-well, clear plastic, tissue culture plates (Falcon 3047). The plates allowed application of treatments and measurement of color and ethylene biosynthesis in individual discs with minimal disturbance. Plates of discs were stored in boxes flushed with three volumes of water-saturated air per hour under isothermal conditions (20°C), and removed only for disc treatment and measurement.

Pericarp discs contain at least three distinct tissues: exocarp, endocarp, and vascular tissues. The exocarp is 1 to 2 mm thick and lies immediately beneath the transparent cuticle. It is composed of relatively small, densely packed cells, is rich in pigments, and is the first pericarp tissue to undergo color change during normal ripening. The endocarp extends inward from the exocarp to the locule. Endocarp cells are larger, less densely packed, lightly colored, and slower to redden. Vascular tissues lie within the endocarp and are the last pericarp tissues to redden during normal ripening (23).

Measurement of Color and Ethylene

The color of individual discs was measured daily through the clear plastic bottom of the tissue culture plate with a handheld reflectance colorimeter (Minolta CR-200). Discs could be turned to measure the color of either the intact skin (exocarp) or cut flesh (endocarp) surface. Color was recorded using the L*ab* uniform color space (CIELAB), where L* indicates lightness, a* indicates hue on a green (−) to red (+) axis, and b* indicates hue on a blue (−) to yellow (+) axis (18).

Rates of ethylene biosynthesis of individual discs were determined daily or as needed. In a sterile hood, the normal plate lid was replaced by a three-tiered assembly consisting of a sheet of fresh Parafilm, a soft neoprene-lined rubber gasket, and a reinforced perforated plastic lid. Hand clamps compressed the assembly against the raised rim of each well to produce a 3.5 mL gas-tight chamber around each disc. After 15 to 20 min, a 1-mL sample of accumulated gases was drawn from each well with a needle inserted through the gasket and Parafilm, and the sample was analyzed for ethylene as above. Ethylene measurements were made before color measurements or the treatment or turning of discs to minimize induction of ethylene by handling.

Application of Treatments

Treatments were applied as sterile, unbuffered aqueous solutions in aliquots of 10 μL distributed by pipette in several droplets across the cut surface of the endocarp. The droplets were usually absorbed into the disc within an hour after treatment. Pectic oligomers and monomeric galacturonic acid were added to discs at concentrations of 100, 10, or 1 μg/10 μL. Galacturonic acid treatments were applied to account for oligomer effects that might be due to changes in pH or metabolizable substrate. ACC was added at a concentration of 10 nmol/10 μL. ACC treatments were applied to account for oligomer effects that might due to induced ethylene. Treatments were applied twice, the first treatment usually 24 to 48 h after disc excision and the second treatment approximately 24 h later. For study of responses at progressive color stages, first treatments were applied at 1, 2, or 3 d, or at 2, 4, or 6 d, after excision, and second treatments 24 h later. Treatments were applied to sets of eight replicate discs.

The pectic oligomers used in this study consisted of mixtures of smaller (G7) and larger (G12) homooligomers of α-1,4-D-galacturonic acid prepared by Mike Saxton, University
Treatment of discs with water alone induced no increase or only a slight transient increase in the rate of ethylene biosynthesis, usually less than 25% of that induced by 100 μg of G7 or G12 (Fig. 2). Treatment with galacturonic acid usually induced a slight, transient increase in the rate of disc ethylene biosynthesis that was typically 25 to 50% of that induced by an equal weight of pectic oligomers (Fig. 2).

Application of ACC to the cut surface of pericarp discs resulted in a rapid, predictable conversion of ACC to ethylene, which reached a maximum rate usually within 2 h. Both the maximum rate and total yield of ethylene from ACC depended on the concentration of ACC applied (data not shown). Treatment with 10 nmol of ACC yielded ethylene at more than twice the maximum rate and four times the yield of ethylene induced by a 100 μg treatment with pectic oligomers, in 1 d old discs from fruit at maturity stages MG2, MG3, and MG4 (Fig. 2). Treatment with 10 nmol of ACC was used as a test of ethylene effects on reddening and darkening responses.

Effect of Maturity on Ethylene Biosynthesis

Pectic oligomers induced a similar, short-term increase in ethylene biosynthesis in discs from fruit at all maturity stages, MG2 (Fig. 1a), MG3 (not shown), and MG4 (Fig. 1b), however, the pattern of decline after the induced maximum changed with fruit maturity. When discs from MG2 fruit were treated with oligomers, the rate of ethylene biosynthesis usually returned to the low, stable levels of the untreated control discs within 24 h after treatment (Fig. 1a). When discs from MG3 or MG4 fruit were treated, the induced increase in ethylene biosynthesis was frequently superimposed over the climacteric rise in ethylene biosynthesis, which usually began within one or two days after excision of discs from MG3 or MG4 fruit and coincided with reddening of the pericarp discs (Fig. 1b) (7). After oligomer treatment of discs from MG3 or MG4 fruit, ethylene biosynthesis often did not return to the level of the water-treated controls until several days after treatment.

RESULTS

Transient Change in Ethylene Biosynthesis

Pectic oligomers induced a rapid, transient increase in the rate of ethylene biosynthesis when applied to tomato pericarp discs. Ethylene biosynthesis rose within an hour of treatment, reached a maximum after about 4 h, then declined slowly toward control levels (Fig. 1). The maximum rate of ethylene biosynthesis generally increased with amount of oligomer applied from 1 to 100 μg oligomer per disc (Fig. 1), and reached a maximum at around 100 μg oligomer per disc. In response to 100 μg of oligomer, the rate of ethylene biosynthesis in preclimacteric discs typically increased 100 to 300% from pretreatment levels, from below 4 nL/g fresh weight/h to above 10 nL/g fresh weight/h. The pattern of increase was similar in response to G7 and G12.

Figure 1. Short-term change in ethylene biosynthesis in response to larger pectic oligomers (G12) by pericarp discs prepared from single green tomato fruit at maturity stages MG2 (a) or MG4 (b). Treatments of water or oligomers in water were applied 24 h after disc excision. Bars indicate the standard deviation for sets of eight pericarp discs.

Figure 2. Short-term change in ethylene biosynthesis in response to larger (G12) or smaller (G7) pectic oligomers, galacturonic acid (G1), or ACC. Pericarp discs were from the same MG4 tomato fruit represented in Figure 1b. Treatment application and statistics as in Figure 1.
frequently after the climacteric peak (Fig. 3). Galacturonic acid did not induce a persistent increase in the rate of biosynthesis in discs from MG3 or MG4 fruit. ACC did produce a long-term increase in ethylene biosynthesis in discs from MG3 or MG4 fruit, but the increase due to added ACC appeared to be less than that expected from conversion of ACC to ethylene (440 nL/disc or 1460 nL/g fresh weight).

**Change in Tissue Reddening**

Pectic oligomers induced an increase in redness and the rate of reddening when applied to tomato pericarp discs (Fig. 4). This increase in redness was more apparent on the cut surface of the endocarp to which treatments were applied (Fig. 4, a and b; Fig. 5), but was usually detectable on the intact exocarp side as well (Fig. 4, c and d). The increase in rate of reddening was usually apparent within 1 d of treatment, and usually continued for several days after treatment, producing a significant increase in the redness of both disc surfaces which usually persisted throughout subsequent color change. The increase was related to dose over at least two orders of magnitude (Fig. 5), and reached a maximum at around 100 μg oligomer per disc. The pattern of increase in redness was generally similar in response to G7 or G12 (Figs. 4 and 5).

ACC also increased the rate of reddening of tomato pericarp discs, but affected the relative reddening of endocarp and exocarp tissues differently than did pectic oligomers. The increase in reddening of the cut surface of the endocarp due to ACC was consistently less than that induced by pectic oligomers (Fig. 4, a and b), whereas the increase in reddening of the intact surface of the exocarp was usually greater than that due to oligomers (Fig. 4, c and d). Treatment with galacturonic acid usually also induced a significant increase in the rate of reddening of both disc surfaces, but this increase
was always less than that induced by an equal weight of pectic oligomers (data not shown).

**Effect of Maturity on Tissue Reddening**

Pectic oligomers induced an increase in reddening of endocarp and exocarp tissues when applied to green discs excised from tomatoes at all maturity stages: MG2 (Fig. 6), MG3 (not shown), and MG4 (Figs. 4 and 5). Discs excised from MG2 fruit did not usually redden in this interval when left untreated or treated with water (Fig. 6); whereas untreated or water-treated discs excised from MG3 or MG4 fruit usually began to redden within one or two days (Figs. 4 and 5). Thus in MG2 discs, added oligomers induced reddening in both tissues, while in MG3 and MG4 discs, they accelerated ongoing changes in redness.

The effect of ACC on disc reddening changed with fruit maturity and differed between endocarp and exocarp tissues. ACC induced an increase in reddening of exocarp tissue in discs from fruit at all green maturity stages; however, exocarp tissue from MG2 fruit reddened the least in response to ACC relative to pectic oligomers (Fig. 6, c and d). ACC induced an increase in reddening of endocarp tissue only in discs from MG3 or MG4 fruit (Fig. 4). Endocarp tissues from MG2 fruit did not usually redden in response to 10 nmol of ACC (Fig. 6, a and b), even though this treatment produced more than four times the total ethylene of red-inducing oligomer treatments. Endocarp reddening and increased exocarp reddening could be induced in MG2 discs by treatment with 50 or 100 nmol of ACC (data not shown), which produced a further 5- to 10-fold increase in total ethylene released.

The effect of pectic oligomers and ACC on color change at later color stages ($a^* > 0$) was determined by treatment of subsets of discs at 1 or 2 days intervals after excision from MG3 and MG4 fruit. The absolute and relative effects of oligomers and ACC on endocarp and exocarp reddening changed as exocarp color progressed from the green ($a^* < 5$) through the pink ($0 < a^* < 10$) stages of rapid color change to light red ($a^* > 10$). At the start of color change ($exocarp a^* < 5$), endocarp reddening was more sensitive to oligomers than to ACC treatment (Fig. 5, a and b). During color change, the reddening response of endocarp tissue to pectic oligomers remained relatively constant, while the color response to ACC increased (Fig. 7, a and b); as a result the color responses of endocarp to oligomers or ACC converged as ripening progressed and exocarp $a^*$ exceeded 5. In contrast, exocarp reddening was more sensitive to ACC than to oligomer treatment at the start of color change (Fig. 4, c and d). During color change, the reddening response of exocarp to oligomers initially increased (exocarp $a^* < 5$), then responses to oligomers and ACC both decreased during the stage of rapid color change, such that neither oligomers nor ACC induced a
consistent increase in reddening of exocarp tissue when a* exceeded 10 (Fig. 7, c and d).

Changes in Endocarp Darkening

Pectic oligomers also induced a second, distinct effect on disc color change: a darkening of the cut surface of the endocarp of green pericarp discs, as indicated by a decrease in the L* value (Fig. 8). A change in darkness of the endocarp was usually apparent within 1 d after oligomer treatment, and usually continued for several days after treatment, producing a significantly darker surface which persisted throughout subsequent reddening. Darkening increased with oligomer concentration, from 1 to 100 μg (data not shown). In some earlier experiments, in which disc desiccation may have been a problem, the darkening of tissues induced by oligomers was pronounced, originating in the vascular strands, then spreading to the cut surface of the endocarp, and was presumed to be due to synthesis of phenolic compounds (8). As disc storage conditions were changed to reduce water loss from the discs (from 5% to 1% per week), the darkening of endocarp in response to oligomers became the slight, uniform darkening of the cut endocarp surface reported here.

Induction of darkening by pectic oligomers was not simply due to induced ethylene, or to addition of a metabolizable substrate, or to buffering of tissue pH. Added ACC did not induce tissue darkening, and an equal weight of added galacturonic acid did not produce equivalent darkening (Fig. 8).

DISCUSSION

Changes in Ethylene Biosynthesis

In tomato fruit, increases in ethylene biosynthesis can be induced by both environmental and developmental events. Wounding of tomato pericarp tissues results in a rapid, transient increase in ethylene biosynthesis at all color stages (19).

Figure 7. Change in color of endocarp and exocarp tissues during ripening in response to smaller (G7) pectic oligomers or ACC applied at progressive ripening stages. Pericarp discs prepared from a single MG4 tomato fruit. Subsets of discs were given first treatments at 2, 4, or 6 d after excision, and second treatments approximately 24 h later, both immediately after color measurement. Color and rate for each subset are shown for the first 2 d after treatment only. Color measurement and statistics as in Figure 4.

Figure 8. Change in lightness of endocarp tissues in response to larger (G12) and smaller (G7) pectic oligomers or ACC. Pericarp discs prepared from a single MG4 tomato fruit. Lightness is expressed using the gray (L*) axis of the L*a*b* color system. Treatments were applied twice, approximately 24 h apart, immediately after measurement. Bars indicate the standard deviation for sets of eight pericarp discs.
The similar rapid increase in ethylene biosynthesis induced by pectic oligomers suggests that pectic oligomers may act as wound signals in pericarp tissues. Pectic oligomers have been shown to induce many other wound responses, such as synthesis of lignins, phytoalexins, and hydroxyproline-rich glycoproteins, in a variety of plant tissues (29).

A role for pectic oligomers in mediating wound responses does not preclude a possible role in regulating climacteric ripening. Indeed, ethylene biosynthesis appears to serve such a dual function in tomato (2, 16, 19, 20). Climacteric ripening is characterized by a slow rise in ethylene biosynthesis (2, 13). The persistent increase in climacteric ethylene induced by pectic oligomers is consistent with a role for endogenous oligomers in the regulation of ripening. In assessing the effects of pectic oligomers on ripening, it is important to distinguish between direct effects of oligomers on ripening processes and secondary effects which are a consequence of induced ethylene.

**Changes in Tissue Reddening**

A change in color of pericarp tissues from green to red is a definitive characteristic of ripening in tomato fruit (2, 13). By this standard, pectic oligomers can both induce and accelerate the rate of ripening of endocarp and exocarp tissues in pericarp discs. However, the mechanisms by which pectic oligomers accelerate the ripening of exocarp and endocarp tissues appear to differ.

In exocarp tissues, at later maturity stages, the acceleration of reddening by pectic oligomers may be simply a consequence of induced ethylene biosynthesis. In endocarp tissues, the acceleration of reddening by oligomers appears to be a more direct effect of oligomers on ripening processes; the effect of pectic oligomers on endocarp reddening can not be duplicated by treatment with ACC, except at the final maturity stages.

**Regulation of Disc Ripening by Ethylene**

Changes in the sensitivity of specific tissues and processes to ethylene may be an important factor in regulation of tomato ripening (20, 21). The reddening response of pericarp tissues to added ACC can serve as a measure of tissue sensitivity to ethylene. By this standard, exocarp and endocarp tissues appear to differ in their sensitivity to ethylene, and to exhibit independent changes in sensitivity to ethylene as ripening progresses. Exocarp tissues appear sensitive to added ACC at all maturity stages, and more sensitive than endocarp tissues from MG2 until exocarp $a^* > 5$ (Figs. 4, 6, and 7). Endocarp tissues appear insensitive to added ACC at the MG2 maturity stage (Fig. 6, a and b), but become sensitive to added ACC by MG3 (Fig. 4, a and b) and increase in sensitivity into the late ripening stages (exocarp $a^* > 10$; Fig. 7, a and b). The tissue-specific sequence of reddening seen in pericarp discs (7) and in intact fruit (23) may result from these initial differences and differential changes in sensitivity to endogenous ethylene concentrations.

Ripening processes can be limited by sensitivity to ethylene or by ethylene concentration (20, 21). In exocarp tissues, both the ethylene sensitivity of exocarp tissues and the ethylene concentration in untreated pericarp discs appear sufficient to allow ripening of exocarp at the rate characteristic of intact fruit (7). In endocarp tissues from MG2 fruit, insensitivity to ethylene appears to limit endocarp response to endogenous ethylene and added ACC. By the onset of exocarp color changes, ethylene concentration appears to replace ethylene sensitivity as the limiting factor in endocarp reddening. The ethylene concentration in untreated pericarp discs appears insufficient to allow a normal rate of reddening in endocarp tissues, and endocarp tissues in discs fail to redden to the extent found in intact fruit (7).

**Regulation of Disc Ripening by Added Pectic Oligomers**

The mechanisms by which pectic oligomers might affect the ripening of endocarp tissues are unknown. We speculate that added oligomers might accelerate ripening by increasing the sensitivity of endocarp tissues to ethylene, and thus allow acceleration of ripening processes by existing ethylene concentrations. In a similar manner, the increase in soluble uronides which occurs in pericarp discs at middle color stages (7) may be responsible for the increase in sensitivity of endocarp tissues to added ACC which occurs at the same stage.

It has been reported that pectic materials generated from tomato fruit cell walls (5) and PG isolated from red tomatoes (1) can induce ethylene biosynthesis and accelerate exocarp reddening when introduced into whole, green fruit by vacuum infiltration. Our results appear consistent with these reports, but also suggest caution in interpreting such responses. Ethylene induction after vacuum infiltration may represent a vascular tissue wound response to pectic oligomers, and subsequent color change may merely be a response to the induced ethylene.

**Are Pectic Oligomers Endogenous Regulators of Ripening?**

The effects of added pectic oligomers on tomato endocarp tissues are consistent with a role for native oligomers in the regulation of ripening processes in both intact fruit and pericarp discs. We suggest that the rate of ripening of endocarp tissues may be regulated, in part, by the release of pectic oligomers from the cell walls of adjacent exocarp tissues. Other carbohydrates released from the wall might play similar or complementary roles. We have found that endogenous carbohydrates isolated from ripening tomato fruit, but low in uronic acids, also induce changes in ethylene biosynthesis and tissue reddening in pericarp discs (unpublished data), a result similar to that recently reported by Tong and Gross (28).

Tiemann and Handa (26) have shown that PG activity spreads through the exocarp of intact tomato fruit, before progressing into adjacent endocarp tissues. Recent work in our laboratory indicates that water-soluble uronides (7) and small pectic oligomers, in particular (V Vreeeland, JW Labavitch, unpublished data) are present in ripening pericarp discs and increase in concentration as the exocarp redens. Thus, pectic oligomers appear to be present at a place and time appropriate to a role in regulation of the tissue-specific sequence of tomato ripening.
LITERATURE CITED