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Influence of heat shocks on the kinetics of chilling-induced ion leakage from tomato pericarp discs

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Abstract

The kinetics of ion leakage from chilled tomato pericarp discs was altered by heat-shock treatments that reduced chilling-induced increases in ion leakage. Pericarp discs (5 mm × 12 mm diameter; 0.52 ± 0.04 g) were excised from mature-green tomato fruit (*Lycopersicon esculentum* Mill. 'Castelmart') and held at 12.5 °C for 18 h before treatment. After a lag of 4 days, the rate of ion leakage into an isotonic 0.2 M mannitol solution increased with increasing duration of chilling at 2.5 °C, reaching a 4.2-fold increase after 14 days. The rate of chilling-induced ion leakage was reduced 64% by a 15 min, 45 °C heat shock applied 1 h before 14 days of chilling. Heat shocks and chilling had no effect of the kinetics of ion leakage from the fast, apoplastic compartment. The apoplastic concentration of ions increased three-fold in non-heat-shocked tissue, while it decreased 50% in heat-shocked tissue over 31 days of chilling. The 6.2-fold increase in leakage from the slow, symplastic compartment following 31 days of chilling was reduced 63% by a 15 min heat shock applied before chilling. The heat-shock treatment protected membrane components involved in ion movement across the membrane from chilling-induced damage, such that ion leakage only increased a fraction of that in non-heat-shocked tissue.
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1. Introduction

Exposure of susceptible plant tissue to non-freezing temperatures below 10–12 °C induces a physiological disorder called chilling injury (Lyons, 1973; Saltveit, 2000). The extent of injury depends on the environment to which the tissue was previously exposed, the type of tissue (e.g. meristematic, vegetative, root, stem, leaf and unripe or ripe fruit), temperature and length of exposure, and post-chilling conditions. Symptoms of chilling injury include increased membrane permeability and a resultant increase in leakage of cellular constituents (Murata, 1990; Sharom et al., 1994). The rate of ion leakage from excised tissue into an isotonic aqueous solution is a useful measure of the severity of chilling-induced increase in membrane permeability (King and Ludford, 1983; Saltveit, 2002).

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Exposure to a number of abiotic stresses before chilling (e.g. cold shock, ethanol, heat shock, osmotic shock, or salinity) increases chilling tolerance (Saltveit, 1991). Heat shocks (i.e., brief exposures to temperatures about 10 °C above the normal growing temperature) induce the preferential de novo synthesis and accumulation of a unique set of proteins called heat-shock proteins that are thought to confer thermotolerance (Vierling, 1991). A 10 min 45 °C heat shock applied before 7 days of chilling at 2.5 °C significantly reduced the rate of chilling-induced ion leakage from tomato pericarp discs (Saltveit, 1991, 2002). Pre-chilling heat-shock treatments that increase chilling tolerance are thought to work through the induced synthesis and accumulation of specific heat-shock proteins (Lafuente et al., 1991; Sabehat et al., 1996). These heat-shock proteins could act as chaperons to protect chilling sensitive components of the cell, e.g. enzymes and membranes (Vierling, 1991). Alternatively, heat shock-induced changes in protein synthesis may protect against symptoms arising from chilling-induced alterations in protein synthesis since heat shock-induced protein synthesis appears to subordinate other stress-induced protein synthesis (Campos-Vargas et al., 2004).

When subjected to a kinetic analysis, leakage data from excised discs of tomato pericarp tissue can be described by the combination of two exponential equations of the form $y = C \times \left(1 - e^{-Kt}\right)$, that model ion leakage from a 'fast' extra-cellular apoplastic reservoir of ions (C_f, K_f; $y = C_f\left(1 - e^{-K_f t}\right)$), and from a 'slow' cellular symplastic reservoir (C_s, K_s; $y = C_s\left(1 - e^{-K_s t}\right)$) (Saltveit, 2002). In these equations, C represents the concentration of ions and K represents rate constants. The leakage model could be described with three components of the analysis: K_s, K_f, and C_f. Since the concentration of ions is expressed as the percent of total conductivity (i.e., $C_f + C_s = 100\%$), determining C_f determines C_s. The apoplastic concentration of diffusible ions (C_f) is much smaller than the symplastic concentration (C_s), and since small changes in C_f are more easily discerned than similar changes in C_s, C_f is the preferred member of the pair to be reported.

Chilling at 2.5 °C increased the rates of ion leakage and the values of C_f and K_s, but had no effect on K_f (Saltveit, 2002). This implies that chilling increased the permeability (K_s) of the cellular reservoir that allowed ions to leak out during the exposure to chilling and increase the content of C_f. However, when ion leakage was measured from aged, non-chilled tissue at temperatures from 2 to 20 °C, C_f remained unchanged and the changes in K_s paralleled those in K_f. These changes in the rate constants were consistent with the effects of temperature on rates of passive diffusion. The abrupt increase predicted by the membrane phase-transition model of chilling injury (Lyons, 1973) was not observed.

If heat-shock treatments confer chilling tolerance by inducing the production of proteins that interact with and stabilize proteins and/or membranes, these changes should be reflected in altered kinetics of chilling-induced ion leakage. Research reported here describes the effects of heat-shock treatments on the kinetics of chilling induced ion leakage from excised discs of tomato pericarp tissue.

2. Materials and methods

2.1. Plant material

Mature-green tomato fruit (*Lycopersicon esculentum* Mill. 'Castelmart') were harvested from the UC Davis, Vegetable Crop Department's field facility. The fruits were washed in dilute sodium hypochlorite (5% aqueous solution of commercial bleach), air-dried in a laminar-flow hood, and pericarp discs aseptically excised with a 12 mm diameter cork borer. The pericarp discs were trimmed of adhering locular tissue to produce 5-mm thick discs (0.52 ± 0.04 g), which were washed three times in deionized water and blotted dry. All procedures were performed under aseptic conditions. Three to four discs from each fruit were put into one sector of a four-sectored plastic 10 mm × 100 mm diameter Petri dish with the epidermis surface down and the top put back on the dish. The dishes were put into 20 cm × 26 cm × 13 cm deep plastic tubs lined with wet paper towels, the top was loosely covered with aluminum foil, and the tubs placed at 12.5 °C for 18 h.

2.2. Application of heat shocks

Tomato pericarp discs were heat-shocked by floating the plastic Petri dishes in a water bath maintained at 45 °C. The dishes were then floated on room temperature water for 1 h before being placed in plastic tubs

lined with wet paper towels. The tubs were loosely covered with aluminum foil, and placed in a 2.5 or 12.5 °C cold room.

2.3. Measurement of chilling injury

After holding at 2.5 or 12.5 °C, the dishes were removed to 20 °C for 1 h before two pericarp discs were put into 20 mL of an isotonic 0.2 M mannitol solution in a 50 mL plastic centrifuge tube. The conductivity of the solution was measured at 0, 15, 30, 60, 120 and 180 min. The tubes were gently shaken between readings. The tubes were then capped, and frozen and thawed twice with shaking over the next few days. The total conductivity of the solution was read after the tubes had come to 20 °C with shaking.

2.4. Statistical analysis

Raw conductivity measurements were converted to percent of total conductivity, and a kinetic analysis of the ion efflux data was done using a series of iterative calculations as previously described (Saltveit, 2002). Linear regressions were also calculated for the change in solution conductivity over time to find the rate of ion leakage. All experiments were replicated at least two times and each treatment had at least five replicates. Means and standard deviations were calculated from the data.

3. Results and discussion

3.1. Ion leakage from chilled tomato pericarp discs

Chilling increased the rate of ion leakage from aged, 5 mm × 12 mm diameter pericarp discs excised from mature-green tomato fruit (Fig. 1). After remaining relatively constant at 1.6 ± 0.4% of total conductivity per hour for 4 days of chilling, the rate of ion leakage increased 1.8-fold to 2.9 ± 0.4, and 2.6-fold to 4.2 ± 0.4 after chilling for 8 and 14 days, respectively. These results differ from those previously reported (Saltveit, 2002) in which pericarp discs excised from the same tomato cultivar exhibited chilling-induced increases in ion leakage after 3 days of chilling at 2.5 °C, and had higher rates of leakage from non-chilled tissue (2.4 ± 0.2% versus 1.6 ± 0.4% total conductivity per hour).

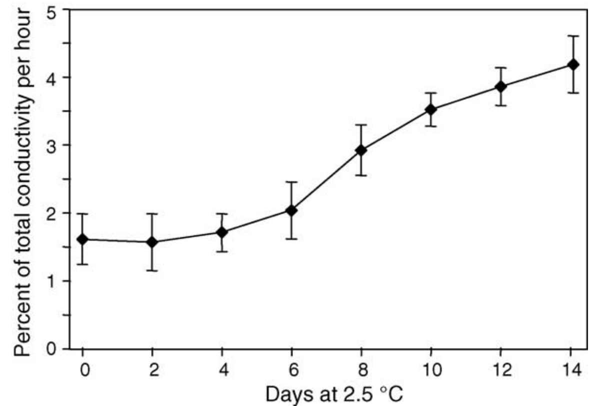

Fig. 1. Effect of chilling on the rate of ion leakage from 5 mm × 12 mm diameter aged pericarp discs excised from mature-green tomato fruit. The vertical bar associated with each mean represents the standard deviation about that mean.

Fruit used by Saltveit, 2002 were harvested during the summer and experienced higher growing temperatures (e.g. >37 °C) than fruit used in the present study that were harvested during the cooler (e.g. 25 °C), fall growing season. Tomato fruit harvested when cool are less chilling-sensitive than fruit harvested when hot (Saltveit, 1991) and differences in the growing temperature may account for the differences in chilling sensitivity seen in these two studies. However, although a longer duration of chilling at 2.5 °C was necessary to induce the same rate of ion leakage as in the previous study, the shape of the resulting efflux curves were similar as were the maximum induced rates of ion leakage.

A kinetic analysis of the efflux data from chilled pericarp discs produced values for the three components of the two exponential equations used to model chilling-induced ion leakage (i.e., K_f, K_s, and C_f). The coefficient describing leakage from the fast, apoplastic compartment (i.e., K_f) remained relatively constant over 24 days of chilling (Fig. 2). In contrast, the coefficient describing leakage from the slow, symplastic component (i.e., K_s) increased 4.3-fold, after remaining relatively constant for the first 4 days of chilling. The fast component (K_f) should remain constant since it represents the passive diffusion of ions from the cell wall and adjacent extra-cellular portions of the tissue. The concentration of ions in the apoplastic space (i.e.,

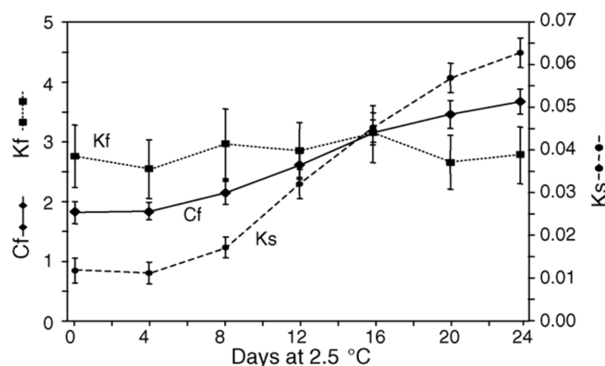

Fig. 2. Effect of chilling on the kinetics of ion leakage from 5 mm × 12 mm diameter aged pericarp discs excised from mature-green tomato fruit. The coefficients of the exponential equations describing ion leakage (i.e., C_f, K_f and K_s) were calculated as described in the text. The vertical bar associated with each mean represents the standard deviation about that mean.

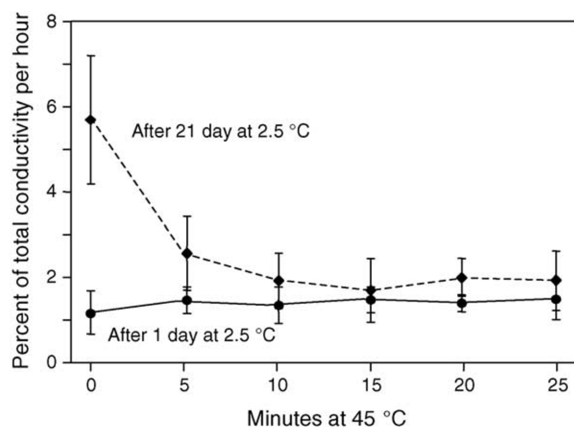

Fig. 3. Effect of heat shock on the rate of ion leakage from 5 mm × 12 mm diameter aged pericarp discs excised from mature-green tomato fruit. The vertical bar associated with each mean represents the standard deviation about that mean.

C_f) increased, as did K_s, but the doubling of C_f was much smaller that the 4.3-fold increase in K_s.

Since the space represented by C_f is not bounded by a semi-permeable membrane that may be affected by chilling, the component representing diffusion of ions from C_f (i.e., K_f) should not change with chilling. However, alterations in resistances to diffusion from C_f (e.g. occlusion of the cell wall matrix), or in ion-binding properties of the cell wall (e.g. more charges sites) could alter K_f. Since K_f remained constant during 24 days of chilling, it is unlikely that significant modifications were induced to the physical properties of the apoplastic portions of the tissue by chilling.

3.2. *Ion leakage from heat shocks and chilled tomato pericarp discs*

Chilling for 21 days at 2.5 °C increased the rate of ion leakage about 4.8-fold, from 1.2 ± 0.5 to 5.7 ± 1.5% of total conductivity per hour (Fig. 3). Applying a 45 °C heat shock to the pericarp discs before chilling significantly reduced chilling-induced ion leakage to levels of tissue chilled for just 1 day (a non-significant level of chilling). There was no visible difference in the discs among the different heat-shock and chilling treatments after removal from chilling and prior to measuring ion leakage (data not shown). The extent, to which chilling-induced ion leakage was reduced by the heat-shock treatment, was similar for 10–25 min of heat shock, and 15 min of heat shock at 45 °C was used in all subsequent experiments.

The duration of time between applying the heat-shock treatment and chilling the tissue did not significantly alter the level of protection conferred by the heat-shock treatment. A 15 min heat shock reduced ion leakage induced by 14 days of chilling by 64%, from 5.8 ± 0.9 to 2.1 ± 0.8% of total conductivity per hour. The same level of inhibition (i.e., 64%) was induced whether the heat shock was applied 0, 3, 9, 15, 21 or 27 h before chilling. Heat-shock treatments are thought to exert their protective effects through the induction, synthesis and accumulation of heat-shock proteins (Lafuente et al., 1991; Sabehat et al., 1996). Since most metabolic reactions, including protein synthesis, proceed at a slower rate in chilled tissue, immediately chilling heat-shocked tissue should have diminished the ability of tissues to synthesize protective heat-shock proteins. However, the lack of a significant effect of holding the heat-shocked tissue for 0–27 h before chilling suggests either that the synthesis of heat-shock-induced proteins may be unaffected by the reduced temperature, that sufficient heat-shock proteins were synthesized even at the slower rate to exert their protective effect, or that the synthesis of heat-shock proteins may not be part of the protective heat-shock effect. Heat-shock proteins are preferentially synthesized in tissue subjected to two abiotic stresses (Campos-Vargas et al., 2004). The heat-shock treatment may sub-

ordinate metabolic reactions induced by chilling that contribute to chilling injury (e.g. production of reactive oxygen species), or the synthesis of proteins that diminish chilling-induced damage (e.g. superoxide dismutase) (Marangoni et al., 1996; Parkin et al., 1989).

3.3. Kinetic analysis of ion leakage from heat-shocked and chilled tomato pericarp discs

The fast apoplastic component of ion leakage (K_f) did not show a significant change during 31 days of chilling, and was not significantly affected by heat-shocking the pericarp discs before chilling (Fig. 4A). In contrast, the concentration of ions in the apoplastic space (i.e., C_f) increased 3.1-fold from 1.8 ± 0.3 to 5.6 ± 0.4 over the 31 days of chilling. This increase was prevented by the heat-shock treatment, such that C_f actually declined 55% from 1.8 ± 0.3 to 0.8 ± 0.4 over the 31 days of chilling (Fig. 4B). After 31 days of chilling, the concentration of ions (C_f) in the apoplastic space of heat-shocked tissue was only 15% of that in non-heat-shocked tissue.

Changes in the slow, symplastic component of ion leakage (K_s) were accompanied by the rise of C_f in non-heat-shocked discs during the 31 days of chilling (Fig. 4C). K_s increased 6.2-fold from 0.011 ± 0.004 to 0.068 ± 0.005 in non-heat-shocked tissue. The heat-shock treatment reduced this 6.2-fold rise in K_s to a 1.3-fold increase from 0.011 ± 0.004 to 0.025 ± 0.005. After 31 days of chilling, the rate of leakage from the slow, symplastic compartment (K_s) of heat-shocked tissue was only 37% of that in non-heat-shocked tissue.

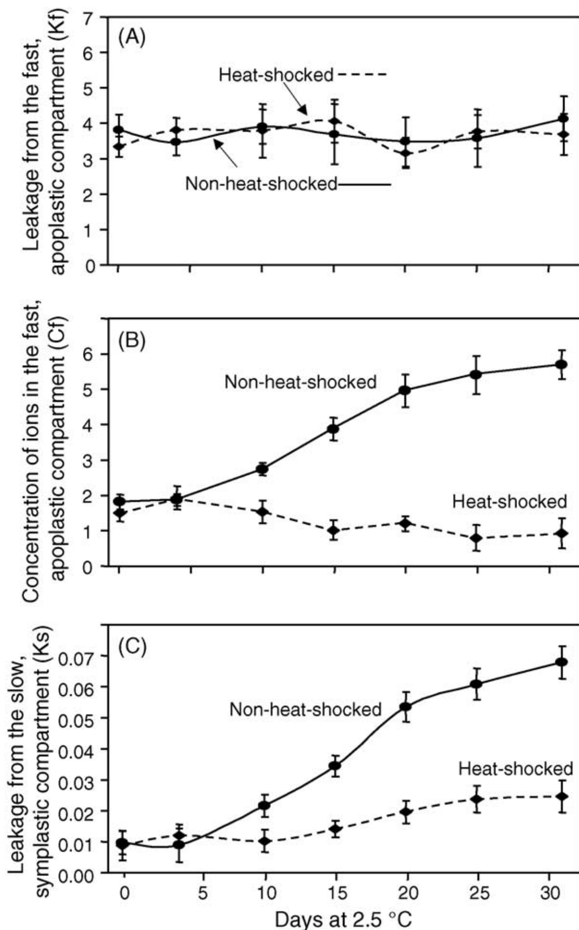

Fig. 4. Effect of chilling and heat shock on the (A) fast, apoplastic compartment (K_f), (B) ion concentration in the apoplastic compartment (C_f), and (C) slow, symplastic compartment (K_s) of the exponential equations describing ion from 5 mm × 12 mm diameter aged pericarp discs excised from mature-green tomato fruit. The vertical bar associated with each mean represents the standard deviation about that mean.

4. Conclusion

The kinetic analysis used in this study was unable to resolve the slow, symplastic component of ion leakage into components representing leakage from the cytoplasm through the plasmalemma and from the vacuole through the tonoplast. The plasmalemma probably represents a major impediment to ion leakage from the cell since solutes must first move through the tonoplast of the vacuole and through the intervening cytoplasm before moving across the plasmalemma into the apoplastic region of the tissue. Relative values for the slow, symplastic component in the ion leakage model (K_s) probably give a good approximation of the change in membrane permeability caused by chilling. (Vickery and Bruinsma, 1973; Simon, 1977).

After a lag of 4–6 days, there was a steady increase in K_s with chilling at 2.5 °C in both heat-shocked and non-heat-shocked tissue. This increase probably reflects an increased permeability of the plasmamembrane/tonoplast. In non-heat-shocked pericarp tissue, this increased permeability was accompanied by a rise in the concentration of ions in the apoplastic space (i.e., C_f), while in heat-shocked tissue C_f actually decreased 55% as K_s increased 130%. The concentration of ions in

the apoplastic space is the sum of ions leaking from the tissue and ions being reabsorbed by the tissue. As chilling progresses, the ability of non-heat-shocked tissue to prevent ion leakage from the tissue and/or to maintain ion reabsorption by the tissue appears to diminish, and ions accumulate in the apoplastic spaces. The heat-shock treatment appears to protect membrane components involved in ion movement across the membrane from chilling-induced damage, such that ion leakage only increased a fraction of that in non-heat-shocked tissue.

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