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The Correlation between Heat-Shock Protein Accumulation and Persistence and Chilling Tolerance in Tomato Fruit[1]

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Heating tomato fruit (*Lycopersicon esculentum*) for 48 h at 38°C prevented chilling injury from developing after 21 d at 2°C, whereas unheated fruit developed high levels of injury. Although the overall protein pattern as seen by Coomassie blue staining was similar from heated and unheated fruit, some high- and many low-molecular-mass proteins were observed in the heated fruit that were absent or present in reduced amounts in unheated fruit. When fruit were injected with [^{35}S]methionine at harvest and then heated, they accumulated high levels of specific radiolabeled proteins that could still be detected after 21 d at 2°C. If the fruit were held at 20°C after heating, the label in the proteins declined rapidly and these fruit were also sensitive to chilling injury. Hsp70 antibody reacted more strongly with proteins from heated and chilled fruit than with proteins from chilled fruit. Hsp18.1 antibody reacted strongly with proteins from heated fruit but not with those from unheated fruit. A 23-kD protein, highly labeled in heated fruit but not in unheated fruit, had its amino terminus sequenced. To our knowledge, this is the first report showing a relationship between the persistence of heat-shock proteins and chilling tolerance in a plant tissue.

Living organisms frequently produce new proteins as part of their response to abiotic environmental stress (Heikkila et al., 1984; Ort et al., 1989; Hightower, 1991). Exposure to nonlethal high temperatures induces the de novo synthesis of a characteristic set of proteins called hsps (Nover et al., 1989). The accumulation of hsps often confers protection against a subsequent heat stress that would have been lethal (Lin et al., 1984; Chen et al., 1988). For example, roots of 3-d-old etiolated soybean seedlings became tolerant to lethal temperature treatments after preincubation at 40°C for 2 h (Chen et al., 1988).

Induction of hsp synthesis also occurs when plants are subjected to thermal stresses similar to those that occur naturally. Chen et al. (1990) exposed pea plants to a gradual increase in temperature up to 40°C and monitored the change in low-molecular-mass hsps. When these plants were allowed to return to their normal growth temperature, the low-molecular-mass hsps that had accumulated in leaf chloroplasts during the heat stress were found to decay with a half-life of around 50 h. The persistence of these hsps suggests that their participation in the recovery process could be as important as their proposed protective role during the stress period itself.

Chilling injury is a physiological disorder that develops in some plants indigenous to the tropics and subtropics (e.g. avocado, cucumber, rice, sorghum, and tomato [*Lycopersicon esculentum*]) when they are exposed to nonfreezing temperatures below 10 to 12°C (Saltveit and Morris, 1990). Prior exposure to heat-shock temperatures has been shown to increase the tolerance of sensitive tissue to subsequent chilling (Lurie and Klein, 1991; Saltveit, 1991). The protection afforded by heat shock against chilling injury in tomato was found to persist for up to 21 d at 2°C. Assuming that hsps are involved to some extent in the ability of heat shock to increase chilling tolerance, the induced hsps would have to persist in the fruit cells during the chilling period. This paper reports on the accumulation of hsps during the heat treatment and their persistence during the chilling period at 2°C as measured by Coomassie blue staining, by radioactive labeling, and by cross-reaction to hsp antibodies.

MATERIALS AND METHODS

Tomato fruit (*Lycopersicon esculentum* cv Daniella) were grown in the greenhouse under normal conditions. Fruit were harvested at the mature green stage and divided into lots of 30 fruit each. In the different experiments, lots were placed directly into 2°C or first held in a 38°C heating chamber for 48 h and then placed at 2°C. In some experiments, heated or unheated fruits were held for 2 or 4 d at 20°C before being transferred to 2°C. After 21 d at 2°C the fruit were transferred to 20°C and the development of chilling injury was monitored. Chilling injury was measured as the appearance of sunken areas on the fruit surface and the development of fungal rots (Lurie and Klein, 1991). The appearance of either of these symptoms caused the fruit to be rated as having chilling injury, and severity (whether one or many loci of damage were present on a fruit) was not measured.

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Abbreviations: 2D, two-dimensional; hsp, heat-shock protein.

Protein Labeling

At harvest, 100 μCi of [^{35}S]Met were injected into the pericarp of several fruits and the area around the site of injection was marked. These fruit were then subjected to the same temperature treatments as the rest of the lot.

Protein Extraction and Gel Electrophoresis

Pericarp tissues were excised, ground in liquid N_2, extracted with Tris-buffered phenol, and precipitated by ammonium acetate in methanol (Hurkman and Tanaka, 1986). When tomatoes were injected with [^{35}S]Met, the area around the injection was excised and treated as above. The precipitate was washed twice with 0.1 M ammonium acetate in methanol and twice with 80% acetone. The pellet was dried and solubilized in a urea buffer (9.5 M urea, 2% [w/v] Triton X-100, 5% [v/v] 2-mercaptethanol, 1% [v/v] Pharmalyte 5/8 [Bio-Rad], and 1% [v/v] Pharmalyte 3/10). IEF gel electrophoresis was performed according to the method of O'Farrell (1975) on a Mini Protean II electrophoresis apparatus (Bio-Rad). The gels were run at 750 V for 3.5 h. IEF gels were extruded, loaded onto a 1-mm-thick 10 to 20% polyacrylamide gradient gel, and run for 1.5 h at 150 V.

For visualization of total proteins, equal amounts of protein were loaded onto the IEF tube gels. Protein concentration was determined by the method of Marousky and Harbaugh (1979). Polypeptides were stained with Coomassie blue reagent (0.25% [w/v] in 50% [v/v] methanol and 10% [v/v] acetic acid) for 30 min and destained with 30% methanol, 10% acetic acid.

For visualization of protein synthesis, samples containing 250,000 cpm were loaded onto the IEF gel tubes. Radioactivity was determined by liquid scintillation counting using Opti-fluor scintillation fluid (Packard Instrument Co., Meriden, CT). Following the second-dimension run, the gel was incubated for 20 min in water and 20 min in 0.5 M salicylic acid, dried, and exposed to x-ray film (X-Omat, Kodak) at −70°C in a cassette with an intensifying screen.

Protein Blotting

For western blotting, equal amounts of protein were loaded onto the IEF tube gels. After running the second dimension, the proteins on the gel were transferred (in 25 mM Tris, 192 mM Gly, 20% methanol, pH 8.3, at 100 V for 1 h) to a nitrocellulose filter using a gel blotter (Bio-Rad). The nitrocellulose filter was blocked with nonfat milk and incubated overnight at 4°C with the primary antibody. Antibody against Hsp70 was used at a 1:2000 dilution, whereas that against hsp18.1 was diluted 1:500. The antigen-antibody complex was detected by alkaline phosphatase reaction. Hsp70 and hsp18.1 antibodies were polyclonal and were generated against proteins from wheat and pea, respectively. The former was a gift from the laboratory of G. Galili (Weizmann Institute of Science, Rehovot, Israel) and the latter was from the laboratory of E. Vierling (University of Arizona, Tucson).

Protein Sequencing

The spot representing the 23-kD protein was excised from the 2D gel and the protein was eluted using a gel eluter (Bio-Rad). Ten such samples were pooled and run again on a one-dimensional polyacrylamide gel. The protein was transferred to a polyvinylidene difluoride membrane (Bio-Rad), and its N-terminal sequence was determined using an automatic sequencing machine (Applied Biosystems 475A Gas Phase Microsequencer System).

RESULTS

Tomato fruit exposed to low temperature (2°C) for 3 weeks developed severe symptoms of chilling injury. Heating the fruit for 48 h at 38°C before moving them to 2°C prevented the development of these symptoms (Table I). When the heat-treated tomatoes were placed for 2 or 4 d at 20°C before being transferred to the low temperature, the protection afforded by the heat treatment remained after 2 d at 20°C, but after 4 d at this temperature the tomatoes were no longer resistant to chilling.

Proteins from heat-treated, heat-treated and chilled, and chilled tomatoes were extracted and analyzed by Coomassie blue staining of a 2D gel. Although the overall patterns appeared similar (Fig. 1), several proteins with molecular masses of 60 to 70 kD were present in heat-treated fruit that were not present in chilled fruit (Fig. 1, arrows and squares). The major differences found between treatments were related to the low-molecular-mass proteins. After 21 d at 2°C, a 23-kD protein was present in heat-treated fruit that was not seen in the extract from chilled tomatoes (Fig. 1, circles). A number of faintly staining polypeptides with molecular masses of 25 to 15 kD were present in heat-treated fruit and not in control fruit (Fig. 1, brackets). There were also three heavily staining polypeptides at 18 kD in heat-treated fruit but only two in unheated fruit (Fig. 1, triangles).

To examine protein synthesis during the heat treatment, tomato fruit were labeled at harvest with [^{35}S]Met and then held for 24 h at 38 or 20°C and the patterns of labeled

Table I. *The effect of different treatments on resistance to chilling stress in tomato fruit*

Fruit were placed for various periods of time at 38 or 20°C before chilling at 2°C for 21 d. Control fruit were placed at 2°C immediately after harvest. Chilling injury was measured after transferring the fruit from the different treatments to 20°C for 5 d. Chilling injury was measured as the appearance of sunken areas on the fruit surface and the development of fungal rots. The experiment was repeated three times with 30 fruit for each treatment, and SD is indicated.

Treatment	2°C, 21 d + 20°C, 5 d Chilling Injury
	% of fruit
Control	86 ± 13.4
4 d at 20°C	88 ± 14.1
1 d at 38°C	8 ± 2.1
2 d at 38°C	4 ± 1.4
2 d at 38°C + 2 d at 20°C	5 ± 2.3
2 d at 38°C + 4 d at 20°C	72 ± 8.6

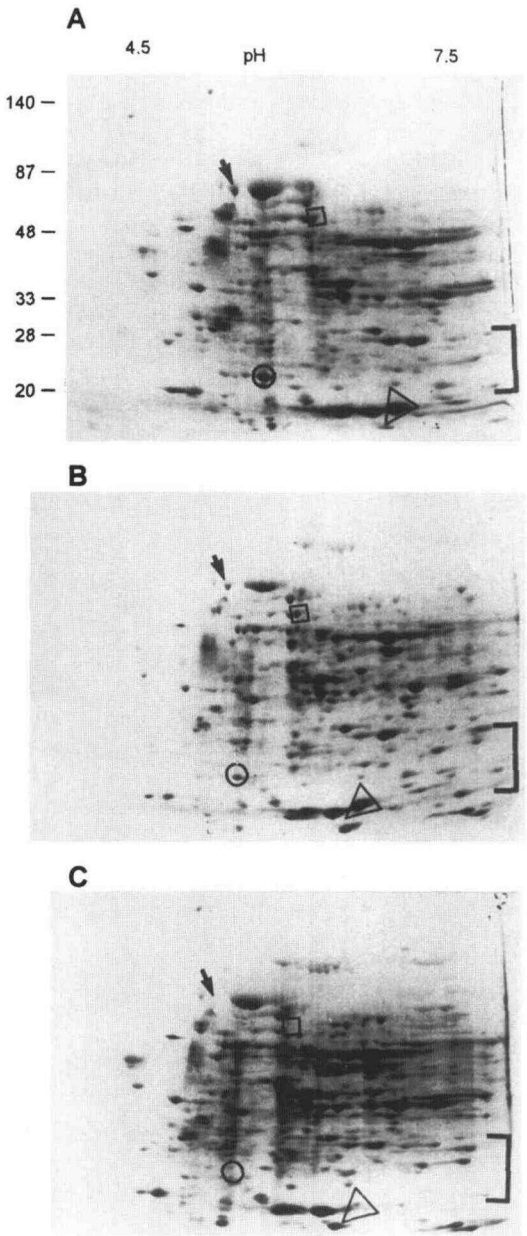

Figure 1. Coomassie blue-stained 2D gels of proteins extracted from mature green tomato fruits exposed to various temperature regimes. A, Forty-eight hours at 38°C; B, 48 h at 38°C + 21 d at 2°C; C, 21 d at 2°C. M_r standards are indicated.

proteins were analyzed by 2D gel electrophoresis. Large differences in protein labeling were observed (Fig. 2). After 24 h at 38°C, proteins with approximate masses of 70 kD, presumably belonging to the hsp70 group, were heavily labeled. In the low-molecular-mass range, there was accumulation of a 23-kD protein (Fig. 2, circles) and two 18-kD proteins (Fig. 2, triangles). In contrast, fruit held at 20°C accumulated only a small amount of labeled 70- and 18-kD polypeptides and did not synthesize the 23-kD protein (Fig. 2B). On the other hand, several heavily labeled proteins that were found in fruit exposed to 20°C were not found in fruits held at 38°C. Proteins that were labeled during the first 24 h at 38°C were also found after 48 h at 38°C (Fig. 3A). However, there was also increased synthesis of several other proteins of low molecular mass after 48 h. After 21 d at 2°C, most of the proteins that were synthesized during the heat treatment were still present (Fig. 3B). In contrast, tomatoes held at 2°C without prior heating accumulated only low levels of the 70-kD protein (Fig. 3C, arrow) and did not accumulate the 23- and the 18-kD proteins (Fig. 3C, circle and triangles, respectively).

To compare the turnover of the proteins synthesized during the heat treatment, tomatoes were transferred for 4 d to 2 or 20°C (Fig. 4). After exposure of heat-treated tomato fruit to 2°C, both the 70-kD and the low-molecular-mass proteins were present. However, at 20°C the label in the hsps declined greatly. The 23-kD labeled protein that was found at a high level after storage at 2°C disappeared almost entirely after exposure to 20°C. If after 4 d at 20°C the heat-treated fruit were placed at 2°C, they were sensitive to chilling (Table I).

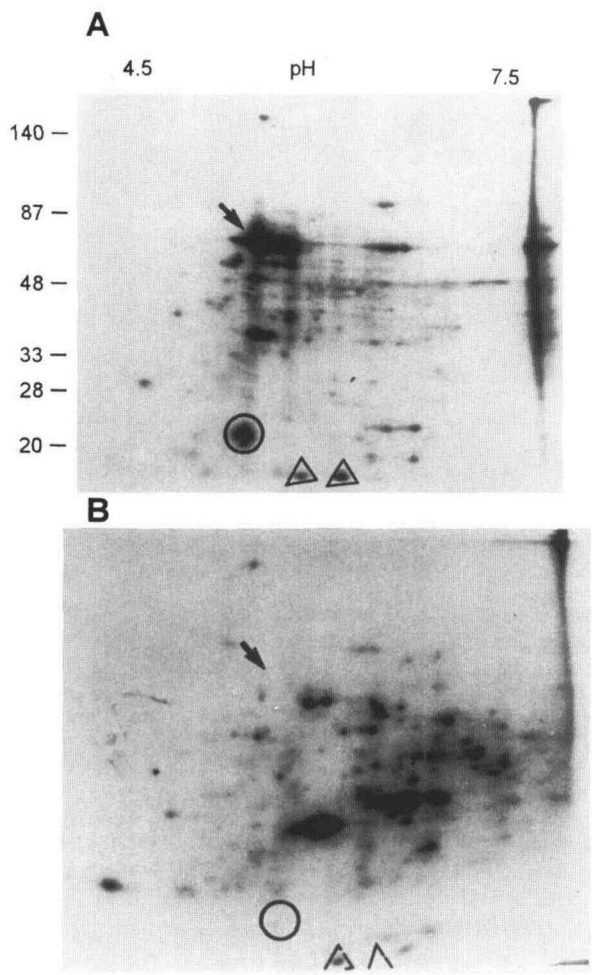

Figure 2. Incorporation of [^{35}S]Met into proteins in whole mature green tomato fruit exposed for 24 h at 38°C (A) or 24 h at 20°C (B). M_r standards are indicated. At harvest, 100 μCi of [^{35}S]Met were injected into the fruit pericarp, and the area around the site of injection was marked.

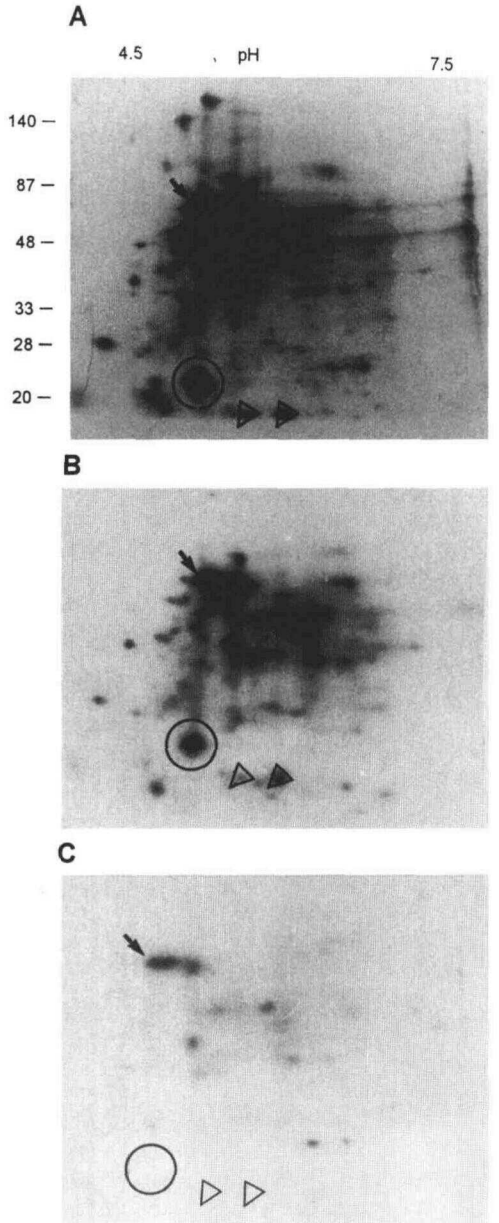

Figure 3. Incorporation of [^{35}S]Met into proteins in whole mature tomato fruit exposed for 48 h at 38°C (A), 48 h at 38°C + 21 d at 2°C (B), or 21 d at 2°C (C). M_r standards are indicated. At harvest, 100 μCi of [^{35}S]Met were injected into the fruit pericarp, and the area around the site of injection was marked.

Antibodies against hsp70 from wheat and hsp18.1 from pea were reacted with proteins from heat-treated and chilled, or just chilled, tomato fruits. A number of polypeptides reacted with the hsp70 antibody in heat-treated and chilled fruits, whereas there was only one polypeptide detected in chilled fruit (Fig. 5, A and B, arrows). Hsp18.1 antibody did not react with proteins from chilled fruit (Fig. 5D) but reacted with at least three polypeptides in the heat-treated and chilled fruit (Fig. 5C, arrows). These results suggest that the 18-kD proteins that were detected by Coomassie blue staining in chilled tomato fruits (Fig. 1C) are antigenically distinct from the 18-kD proteins found in the heated and chilled tomato.

The 23-kD protein was detected by Coomassie blue staining in extracts of heated fruit. This protein was heavily labeled when tomatoes injected with [^{35}S]Met were given the heat treatment and disappeared when the fruits were held at 20°C before chilling. The disappearance of label from the 23-kD protein paralleled the sensitivity of the fruits to chilling injury. The 23-kD protein was purified in sufficient quantity to perform N-terminal sequencing (Fig. 6). We could not find any homology between the partial sequence of the 23-kD protein and any other known protein by screening data banks. In addition, antibodies to hsp21.1 and hsp22.7 of pea did not cross-react with this protein (data not shown). Therefore, this protein does not appear to have been previously isolated from either tomato or other plant tissues.

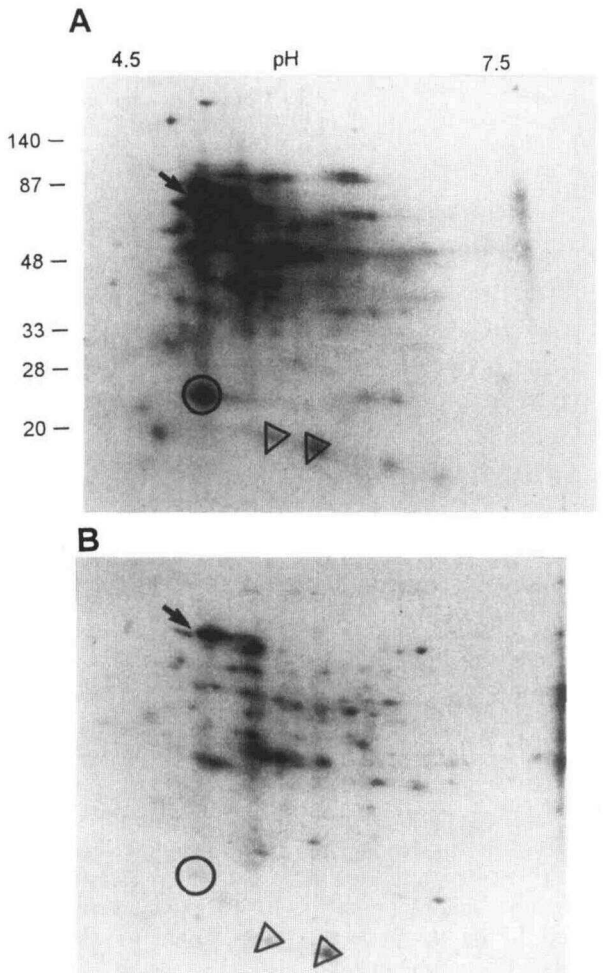

Figure 4. Incorporation of [^{35}S]Met into proteins in whole mature tomato fruit. A, Forty-eight hours at 38°C + 4 d at 2°C; B, 48 h at 38°C + 4 d at 20°C. M_r standards are indicated. At harvest, 100 μCi of [^{35}S]Met were injected into the fruit pericarp, and the area around the site of injection was marked.

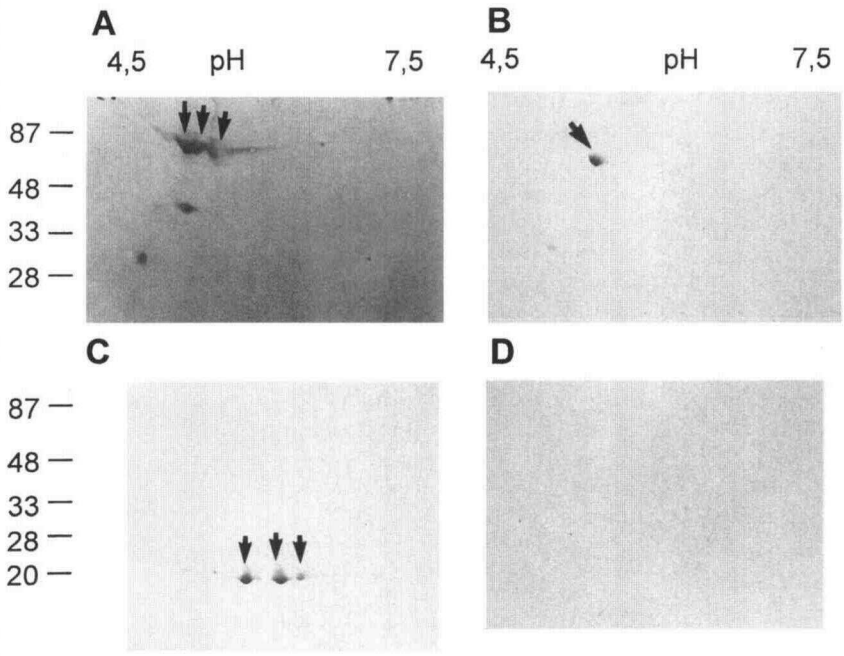

Figure 5. Western blot of 2D gels of proteins extracted from mature green tomato fruits exposed to 2°C for 21 d (B and D) or first heat treated for 48 h at 38°C and then held at 2°C for 21 d (A and C). The filters in A and B were cross-reacted with wheat polyclonal antibody to hsp70 (1:2000 dilution), and those in C and D were cross-reacted with pea polyclonal antibody to hsp18.1 (1:500 dilution). M_r standards are indicated.

DISCUSSION

The results of the present research suggest a correlative relationship between fruit tolerance to chilling temperatures and the continued presence of hsps at low temperature. Members of both the hsp70 family and the low-molecular-mass hsp family (14–25 kD) persisted when the tomatoes were removed from high temperature to chilling conditions, correlating with resistance of the fruit to chilling injury. If fruits were moved from high to ambient temperatures, the level of these hsps decreased markedly during a 4-d period, correlating with the disappearance of fruit resistance to chilling injury. Hsps in other tissues have been found to decay with a half-life of around 50 h at ambient temperature (Chen et al., 1990). Collins et al. (1993) showed that specific proteins are synthesized in heated mung bean hypocotyls, the levels of which decline very slowly when the hypocotyls are held at 5°C. The authors suggested that the persistence of these proteins at low temperature is involved in the protective effect of heat treatment on a chilling-induced increase in electrolyte leakage from the hypocotyls.

The difference in rate of disappearance of hsps when held at 20 or at 2°C may simply be due to decreased metabolism at the low temperature, or there may be a selective retention of the hsps. We addressed this question previously using cultured pear cells labeled with [^{35}S]Met during heat treatment and then held at 25 or 2°C (Ferguson et al., 1994). With one-dimensional gel electrophoresis separation of the proteins after varying times and densitometer scanning, we were unable to measure differences in the rate of decline of labeling in individual hsps. Collins et al. (1993) also showed a gradual disappearance of all the hsps using this method. Therefore, the tentative conclusion is that the persistence of hsps at 2°C compared to 20°C is due to decreased metabolism at low temperature. However, a study of particular hsps and their decline using specific antibodies should be performed before this conclusion can be stated with certainty.

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
N	T	N	T	Q	M	T	A	Y	D	Q	D	D	R	G	U

Figure 6. The first 16 amino acids from the N terminus of the 23-kD protein isolated from heat-treated and chilled fruit.

The present study shows that the hsps accumulate to levels that are detectable not only by labeling methods but also by Coomassie blue staining. Kato et al. (1993) have reported this with heated tomato fruit. However, Coomassie blue staining of proteins is not sensitive enough to detect small changes in the steady-state levels of proteins: the Coomassie blue-stained gels showed only a small difference in the number of polypeptides and intensity of staining of hsp70 from heat-treated and chilled or chilled-only fruit, whereas by using antibody against hsp70, the levels of these proteins were seen to be much higher in the heat-treated and chilled fruit. Equally misleading were the Coomassie blue-stained gels in the low-molecular-mass area, whereby chilled fruit and heat-treated and chilled fruit appeared to accumulate the same 18-kD proteins. When [^{35}S]Met labeling or cross-reaction with hsp 18.1 antibody was used, these proteins were seen to be different.

Several theories have been advanced to account for the nature of chilling injury. The main one, which is restated periodically with modifications, is that the primary lesion is at the membrane level (Lyons, 1973). In many plant tissues, correlations between low-temperature injury and increased electrolyte leakage have been found (Inaba and Crandall, 1988; Saltveit, 1991), and these were also observed in tomato fruit (Lurie and Klein, 1991). This increase is an indication of an alteration in membrane properties.

Elevated temperature also enhances solute and electrolyte leakage (Lurie and Klein, 1990), and the thermotolerance conferred by hsps has been partially attributed to the association of hsps with membranes. Both low-molecular-mass hsps and members of the hsp70 family co-sedimented with plasma membrane in Suc-gradient centrifugations (Cooper and Ho, 1987). It is possible that these hsps become associated with membranes as a result of heat-induced changes in cellular architecture and aid in maintaining normal membrane-associated processes during heat stress (Ho and Sachs, 1989). Prior treatment with high temperature to induce hsp synthesis was found to preserve plasma membrane structure (Lin et al., 1985), diminish solute leakage, and gave rise to the association of a 15-kD hsp with the plasma membrane (Mansfield et al., 1988).

The hsp70 family belongs to a group of proteins known as molecular chaperones (Ellis and van der Vies, 1991) that transiently interact with a wide variety of other cellular proteins. One of the functions of chaperones is to stabilize protein conformation and prevent or disrupt protein aggregates under stress conditions (Vierling, 1991). Hsps may associate with membrane proteins and stabilize their structure and function during and after a heat stress. Low-molecular-mass hsps also appear to have this protective effect. Jinn et al. (1989) found that in vitro low-molecular-mass hsps prevented aggregation of extracted proteins during a high-temperature incubation. Recently, Lee et al. (1995) demonstrated association of hsp18.1 and hsp17.7 (class I and class II hsps) into discrete, 12-subunit, high-molecular-mass complexes. Both complexes were able to prevent inactivation of citrate synthase at elevated temperatures. This protective effect on cytosolic or membrane-associated proteins may be one manner in which hsps confer chilling tolerance on tissue. Hsps in tomato fruits may act in a similar way, and their continued presence at low temperatures suggests their importance in the acquisition of chilling tolerance. We found three main groups of hsps in tomato fruit, with bands at 70, 23, and 18 kD. The 70- and the 18-kD proteins were found to be immunologically related to the well-characterized hsp70 and hsp18.1 (Vierling, 1991). The 23-kD protein could not be identified as any previously reported protein from its N-terminal sequence. However, sequences of a few peptides derived from the protein must be compared to the data base before it can be declared a novel hsp. This work is in progress.

Hsps are up-regulated by elevated temperatures, and their mRNAs are usually most abundant during the first few hours of heat shock and then decline (Vierling, 1991). When plant tissue is transferred from high to ambient temperature the mRNA of hsps declines rapidly (Vierling, 1991). In spinach, the mRNA level of the 70-kD heat-shock cognate gene accumulates during a cold-acclimation period, but the corresponding protein maintains a steady-state level and does not increase (Anderson et al., 1994). We have previously found that tomato fruit heated and then chilled maintains a high level of mRNA for both hsp70 and hsp17 for up to 21 d at 2°C (Lurie et al., 1992). Thus, we suggest that heat treatment induces an increase in both mRNA and protein levels of different hsps in tomato fruit, and that these levels remain high for several weeks at low temperature.

This finding has practical implications for fruit storage. A treatment that induces the accumulation of sufficient hsps in subtropical fruits, such as tomatoes, would allow them to be stored at lower temperatures than is currently feasible. This would extend the storage possibilities of these products, since the temperatures currently used to avoid causing chilling injury are too high to prevent either ripening in climacteric fruits or senescence in nonclimacteric fruits. In summary, to our knowledge the present study is the first in which hsps were closely monitored during heat treatment and subsequent chilling. The results suggest that these proteins may play a role in heat-treatment-mediated protection of fruits against low-temperature injury.

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LITERATURE CITED

Anderson JV, Li QB, Haskell DW, Guy CL (1994) Structural organization of the spinach endoplasmic reticulum-luminal 70-kilodalton heat shock cognate gene and expression of 70-kilodalton heat shock genes during cold acclimation. Plant Physiol **104:** 1359–1370

Chen Q, Lauzon LM, DeRocher AE, Vierling E (1990) Accumulation, stability and localization of a major chloroplast heat-shock protein. J Cell Biol **110:** 1873–1883

Chen YR, Chou M, Ren SS, Chen YM, Lin CY (1988) Observations of soybean root meristematic cells in response to heat shock. Protoplasma **144:** 1–9

Collins GG, Nie X, Saltveit ME (1993) Heat shock increases chilling tolerance of mung bean hypocotyl tissue. Physiol Plant **89:** 117–124

Cooper P, Ho THD (1987) Intracellular localization of heat shock proteins in maize. Plant Physiol **84:** 1197–1203

Ellis RJ, van der Vies SM (1991) Molecular chaperones. Annu Rev Biochem **60:** 321–347

Ferguson IB, Lurie S, Bowen JH (1994) Protein synthesis and breakdown during heat shock of cultured pear (*Pyrus communis* L.) cells. Plant Physiol **104:** 1429–1437

Heikkila JJ, Papp JET, Schutz GA, Bewley JD (1984) Induction of heat shock messenger RNA in maize mesocotyls by water stress, abscisic acid, and wounding. Plant Physiol **76:** 270–274

Hightower LE (1991) Heat shock, stress proteins, chaperones, and phytotoxicity. Cell **66:** 191–197

Ho THD, Sachs MM (1989) Stress induced proteins: characterization and the regulation of their synthesis. *In* PK Stumpf, EE Conn, eds, The Biochemistry of Plants, A Comprehensive Treatise, Vol 15. Academic Press, New York, pp 347–377

Hurkman WJ, Tanaka CK (1986) Solubilization of plant membrane proteins for analysis by two-dimensional gel electrophoresis. Plant Physiol **81:** 802–806

Inaba M, Crandall PG (1988) Electrolyte leakage as an indicator of high-temperature injury to harvested mature green tomatoes. J Am Soc Hortic Sci **113:** 96–99

Jinn TL, Yeh YC, Chen YM, Lin CY (1989) Stabilization of soluble proteins in vitro by heat shock proteins enriched ammonium sulfate fraction from soybean seedlings. Plant Cell Physiol **30:** 463–469

Kato S, Yamagashi K, Tatsuzawa F, Suzuki K, Takano S, Eguchi M, Hasegawa T (1993) Identification of cytoplasmic and nuclear low molecular weight heat shock proteins in tomato fruit. Plant Cell Physiol **34:** 367–370

Lee GL, Polaka N, Vierling E (1995) Structure and *in vitro* molecular chaperone activity of cytosolic small heat shock protein from pea. J Biol Chem **270:** 10432–10438

Lin CY, Chen YM, Key JL (1985) Solute leakage in soybean seedlings under various heat shock regimes. Plant Cell Physiol **26:** 1493–1498

Lin CY, Roberts JK, Key JL (1984) Acquisition of thermotolerance in soybean seedlings. Plant Physiol **74:** 152–160

Lurie S, Klein JD (1990) Heat treatment in ripening apples: differential effects on physiology and biochemistry. Physiol Plant **78:** 181–186

Lurie S, Klein JD (1991) Acquisition of low-temperature tolerance in tomatoes by exposure to high-temperature stress. J Am Soc Hortic Sci **116:** 1007–1012

Lurie S, Watkins C, Ross G, Klein JD, Ferguson I (1992) Prestorage heat treatment to extend storage of tomatoes. Acta Hortic **343:** 128–130

Lyons JM (1973) Chilling injury in plants. Annu Rev Plant Physiol **24:** 445–466

Mansfield MA, Lingle WL, Key JL (1988) The effect of lethal heat shock on nonadapted and thermotolerant root cells in *Glycine max* L. Ultrastruct Mol Struct Res **99:** 96–105

Marousky FJ, Harbaugh BK (1979) Interaction of ethylene, temperature, light and CO_2 on leaf and stipule abscisson and chlorosis in *Philodendron sandens*. J Am Soc Hortic Sci **104:** 876–880

Nover L, Scharf K, Neumann D (1989) Plant Response to Heat Stress and other Stresses. Springer Verlag, Berlin

O'Farrell PH (1975) High resolution two-dimensional electrophoresis of proteins. J Biol Chem **250:** 4007–4021

Ort DR, Marino S, Wise RR, Kent J, Cooper P (1989) Changes in protein synthesis induced by chilling and their influence on the chilling sensitivity of photosynthesis. Plant Physiol Biochem **27:** 785–793

Saltveit ME (1991) Prior temperature exposure affects subsequent chilling sensitivity. Physiol Plant **82:** 529–536

Saltveit ME, Morris LL (1990) Overview of chilling injury of horticultural crops. *In* CY Wang, ed, Chilling Injury of Horticultural Crops. CRC Press, Boca Raton, FL, pp 3–15

Vierling E (1991) The roles of heat shock proteins in plants. Annu Rev Plant Physiol Plant Mol Biol **42:** 579–620