ABSTRACT

Endogenous abscisic acid levels and induced heat shock proteins were measured in tissue exposed for 6 hours to temperatures that reduced their subsequent chilling sensitivity. One-centimeter discs excised from fully expanded cotyledons of 11-day-old seedlings of cucumber (Cucumis sativus L., cv Poinsett 76) were exposed to 12.5 or 37°C for 6 hours followed by 4 days at 2.5 or 12.5°C. Ion leakage, a qualitative indicator of chilling injury, increased after 2 to 3 day exposure to 2.5°C, but not to 12.5°C, a nonchilling temperature. Exposure to 37°C before chilling significantly reduced the rate of ion leakage by about 60% compared to tissue exposed to 12.5°C before chilling, but slightly increased leakage compared to tissue exposed to 12.5 or 37°C and held at the nonchilling temperature of 12.5°C. There was no relationship between abscisic acid content following exposure to 12.5 or 37°C and chilling tolerance. Five heat shock proteins, with apparent molecular mass of 25, 38, 50, 70, and 80 kilodaltons, were induced by exposure to 37 or 42°C for 6 hours, and their appearance coincided with increased chilling resistance. Heat shock treatments reduced the synthesis of three proteins with apparent molecular mass of 14, 17, and 43 kilodaltons. Induction of heat shock proteins could be a possible cause of reduced chilling injury in tissue exposed to 37 or 42°C.

Many plants indigenous to the tropics and subtropics suffer chilling injury upon exposure to nonfreezing temperatures below 12°C (23). Exposure of chilling sensitive tissue to nonchilling temperatures slightly above the chilling temperature or to temperatures above 30°C, treatments called ‘temperature conditioning,’ reduces injury caused by subsequent holding at chilling temperatures (22, 23, 26).

Exogenous application of ABA to cucumber seedlings prior to chilling significantly reduced the chilling-induced increase in ion leakage, possibly by reducing the effects of chilling-induced water stress or the direct effect of low temperature (20). Rikin et al. (19) showed that exogenous application of ABA and pretreatments with physiological stresses, which increased the endogenous level of ABA, increased the tolerance of tissue to subsequent chilling. Stressful temperatures can alter the internal water status and thereby ABA production (25), or they can directly affect ABA levels (4). However, this conclusion was questioned by Eamus and Wilson (6) who showed that increased levels of endogenous ABA did not accumulate if leaves of Phaseolus vulgaris were chilled in a water-saturated atmosphere.

Thermal stress also induces the synthesis of specific HSPs in a wide range of plants (1, 3, 10), and reduces the synthesis of some normally expressed proteins (1). The physiological basis by which these proteins confer thermal tolerance is still unclear, although their synthesis and accumulation appears to provide thermal tolerance, and their selective localization appears to be linked to the expression of this tolerance (10). Lin et al. (13) found that the synthesis of HSPs prevented heat shock-induced leakage from cell. However, the possible relationship between the induction of HSPs and the effect of temperature conditioning on the susceptibility of the tissue to chilling injury was not investigated. HSPs can also be synthesized in response to water stress and ABA (8, 10).

We undertook to study the effect of prior temperature exposure on the susceptibility of cucumber plants to chilling injury and to determine the physiological basis for the observed changes. Since ABA may have a beneficial effect on reducing chilling injury, and ABA, water stress, and thermal stress elicit HSPs which reduce heat shock-induced ion leakage, we evaluated changes in ABA levels and induction of HSPs after exposure to temperatures in a water saturated atmosphere. This was done to determine the possible role of endogenous ABA and HSPs in increasing the chilling tolerance to plants previously subjected to various temperatures. The connection between ABA levels and HSPs was also studied.

1 Present address: Instituto de Agroquimica y Tecnologia de Alimentos. C.S.I.C., C/ Jaime Roig 11, 46010 Valencia, Spain. Financial support was provided by a grant from Generalitat Valenciana Conselleria de Cultura, Educacion i Ciencia.
2 Present address: Departamento de Bioquimica Vegetal. Estacion Experimental del Zaidin. C.S.I.C., 18008 Granada, Spain.
3 Present address: Department of Plant Science, University of Alberta, Edmonton, Alberta, Canada T6G 2P5.
4 Abbreviations: HSP, heat shock protein; TBS, trizma base buffer; PVPP, polyvinylpolypyrrolidone.
MATERIALS AND METHODS

Plant Material and Tissue Preparation

Cucumber seeds (Cucumis sativus L., cv Poinsett 76; obtained from Peto Seed Co., Woodland CA) were imbibed overnight in aerated deionized water and sown in moist vermiculite in 10-cm deep plastic trays. Seedlings were raised in a growth room maintained at 25°C and 60% RH under continuous light provided by cool-white fluorescent tubes giving a photon fluence rate of 70 μE m⁻² s⁻¹ at plant level. One-centimeter discs were excised from fully expanded cotyledons of 11-d-old seedlings with a stainless steel cork borer, and six or eight discs were placed abaxial side down in three sections of a four-sectioned, 15 × 100 mm diameter plastic Petri dish. Each dish contained three replicates of six or eight discs each. The fourth section of the dish contained a quarter section of Whatman No. 1 filter paper moistened with 1-mL water, and a moistened 9-cm diameter Whatman No. 1 filter paper was placed on top of the raised plastic dividers separating the four sections. To further minimize water loss, the dishes were placed in plastic trays with moistened paper towels and the trays loosely covered with thin plastic film. Trays were held in humidified, ethylene-free air overnight at 25°C to allow dissipation of the wound response, and during subsequent experimental procedures.

Temperature Conditioning

Discs were conditioned by exposure to 12.5 or 37°C for 6 h before transfer to either 12.5 or 2.5°C for 4 d. Control dishes were exposed to 12.5°C, the lowest nonchilling temperature, to minimize aging. A holding temperature of 2.5°C was used to induce chilling injury. Thus, tissue discs were subjected to the following four temperature treatments: (a) conditioned at 12.5°C for 6 h then held at 12.5°C for 4 d, (b) conditioned at 12.5°C for 6 h then held at 2.5°C for 4 d, (c) conditioned at 37°C for 6 h then held at 12.5°C for 4 d, and (d) conditioned at 37°C for 6 h then held at 2.5°C for 4 d. Discs were also exposed to 25°C for 6 h (the normal growth temperature) or to 42°C for 6 h in the HSP induction experiments. The 42°C temperature was used since it induces HSPs in a large number of plants (10). Weight loss by the discs during the 6 h conditioning treatments or during the 4 d subsequent holding was less than 2%, and was not significantly different among any of the treatments.

Application of Exogenous ABA

Ethanolic solutions of ABA (184 μM ABA in 0.16 and 1.6% ethanol, and 1844 μM ABA in 1.6% ethanol) were applied as three 0.3 mL drops to the adaxial surface of each 1-cm cotyledonary disc prior to holding at 12.5 or 2.5°C for 6 d. Controls were treated with water, 0.16 or 1.6% ethanol solutions, or left dry. The solutions were filtered through a 0.2 micron filter prior to application.

Measurement of Ion Leakage

Increased rates of passive leakage from sensitive tissue is used as a measure of the alteration of membrane permeability caused by chilling temperatures (9, 26). Measurement of ion leakage can therefore be used to study the effectiveness of treatments that alter the susceptibility of sensitive plants to chilling-induced injury (20, 23, 29).

Dishes from each treatment were held at 20°C for 0.5 h before measuring ion leakage. Discs were incubated in 10 mL of 0.3 m mannitol in 50-mL plastic centrifuge tubes. The tubes were shaken at 120 cycles per min and the conductivity of the solutions was measured after 0.5 and 2.5 h with an Exttech model 480 digital conductivity meter. Preliminary experiments showed that the rate of ion leakage was constant from 0.5 to 4 h. Tubes containing the mannitol solution and the tissue were weighed and heated to boiling for 5 min. After cooling to room temperature with shaking, deionized water was added to make their initial weight, and the total conductivity was measured after an additional 0.5 h of shaking. Ratios of ion leakage are expressed as percentage of the total conductivity per hour (21).

ABA Extraction and Immunoassay

Eight discs of cotyledon tissue (about 400 mg fresh wt), conditioned and held as described above, were immediately frozen in liquid nitrogen after treatment and stored at −40°C until analyzed. Frozen material was thawed and macerated for 5 min in the dark with 5 mL ice-cold methanol containing 0.5 g/L citric acid and 100 mL/L of the antioxidant butylated hydroxytoluene in an Omni-Mixer. Extracts were centrifuged at 2000g, and the supernatant was dried under vacuum at 38°C. Dried samples were diluted in three serial dilutions in ice-cold TBS (6.05 g Tris, 0.20 mg MgCl₂, and 8.8 g NaCl per L, at pH 7.8) and, if the solutions were cloudy, centrifuged at 2000g before immunoassay. Three samples were analyzed for each sample dilution that was within the linear range of the ABA standard curve by the indirect ELISA proposed and verified by Walker-Simmons (24). The ABA-BSA-(4, conjugate) that was provided by Walker-Simmons had been prepared by the method of Weiler (27) with some modification (18).

In Vivo Labeling and Extraction of Labeled Proteins

After weighing, the cotyledon discs were exposed to 12.5, 25, 37, and 42°C for 2 h. The six discs (about 300 mg) were then incubated in 50 mm Tris-HCl buffer at pH 7.8 containing 70 μCi/mL trans-[35S]-labeled methionine (about 70% L-methionine, 15% L-cysteine; 1003 Ci/mmol, ICN, Radiochemicals; Irvine, CA) at the same conditioning temperatures for an additional 4 h with gentle shaking at 90 cycles min⁻¹. The incubation medium was removed and the tissue rinsed twice with 1 mm nonradioactive incubation medium and once with deionized water. Other groups of discs conditioned for 6 h at 12.5 or 37°C, and labeled and rinsed as above were held for an additional period of 4 d at 2.5° or 12.5°C before extraction of soluble proteins. The discs were gently dried and homogenized in 2 mL of ice-cold 62.5 mm Tris-HCl at pH 6.8 containing 1 mm PMSF and 25 mg PVPP. The homogenates were filtered and the supernatant divided into 250 μL aliquots, frozen immediately in liquid nitrogen, and kept at −40°C until used for protein analysis, for the determination of radio-
active amino acid incorporation, or for gel electrophoresis. Protein content was determined according to Bradford (2) with BSA as the standard.

**Analysis of the Incorporation of Radioactive Methionine and SDS Gel Electrophoresis of Proteins**

Incorporation of [14C]amino acids into protein was determined by TCA precipitation as described by Mans and Novelli (15). Soluble protein fractions containing 6 × 10^4 cpm of TCA-insoluble radioactivity were solubilized with 4% SDS, 2% mercaptoethanol, 20% glycerol, and 100 mM Tris HCl (pH 8.0), heated at 95°C for 5 min, and the insoluble material pelleted by centrifugation at 14,000g for 10 min. Lipids and pigments were removed by precipitating the proteins with chloroform and methanol (28). The vacuum-dried pellets were suspended in Laemmli buffer (12) and heated at 95°C for 5 min. Insoluble material was again precipitated by centrifugation at 14,000g for 10 min. Samples were loaded on 13% acrylamide resolving gel overloaded with a 4% acrylamide stacking gel. Proteins were separated by SDS gel electrophoresis according to Laemmli (12). Gels were fixed and fluorographed by treating them with Enhance (New England Nuclear), dried and exposed to Kodak XAR-5 film with a DuPont intensifying screen at −70°C. Molecular mass determinations were carried out by coelectrophoresis with protein standards.

**RESULTS**

**Effect of Temperature Conditioning on Ion Leakage**

The kinetics of ion leakage from 1-cm diameter cucumber cotyledon discs conditioned at 12.5 or 37°C for 6 h and held at 12.5 or 2.5°C for up to 8 d is shown in Figure 1. Despite slight differences in the rate of ion leakage among experiments, the rate of ion leakage was always considerably lower from discs held at 12.5°C than from discs held at 2.5°C. The rate of ion leakage remained relatively constant during the 8 d of holding at 12.5°C (12.5/12.5 and 37/12.5), whereas it steadily increased with time of holding at 2.5°C (12.5/2.5 and 37/2.5).

Temperature conditioning affected the susceptibility of the tissue to chilling (Fig. 1). Leakage from tissue held at 2.5°C was about three times that of leakage from tissue held at 12.5°C after 2 d of treatment, and over eightfold higher after 8 d. The effectiveness of the 37°C exposure to reduce chilling susceptibility was shown by the similar rates of ion leakage from these discs held 2.5 and 12.5°C at 2 d. Even after 8 d, discs exposed to 37°C for 6 h and chilled had rates of ion leakage about half of that from discs exposed to 12.5°C and chilled.

The rate of ion leakage from cotyledon discs conditioned at 12.5, 25, 37, and 42°C for 6 h were not significantly different after 4 d at 12.5°C (Table I). Ion leakage was about eightfold greater from tissue exposed to 12.5 or 25°C and held at the chilling temperature of 2.5°C for 4 d. Exposing the cotyledon discs to 37 or 42°C before holding at 2.5°C for 4 d reduced the rate of ion leakage by almost 90% to the level of nonchilled tissue (Table I; Fig. 1).

**Effect of Exogenously Applied ABA on Chilling-Induced Ion Leakage**

Since ABA is only slightly water-soluble, it was necessary to use ethanol as a bridging solvent (Table II). Since alcohols are known to perturb cellular membranes (21), the results obtained from ABA treated discs were compared to those obtained from discs treated with water containing the lowest level of ethanol necessary to dissolve the ABA.

After 6 d of holding at 12.5°C, the rate of ion leakage was significantly higher at the 5% level from discs treated with the 0.16 and 1.6% ethanol solutions than from discs treated with water or no solution (Table II). After chilling, however, the rate of ion leakage from discs treated with any ethanolic

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**Table I. Effect of Prior Temperature Exposure on Ion Leakage**

Discs (1 cm) were excised from fully expanded cotyledons from 11-d-old cucumber seedlings and exposed to 12.5, 25, 37, or 42°C for 6 h before holding them at 12.5 or 2.5°C for 4 d. Ion leakage was measured as the increase in conductivity in 10 mL of a 0.3 mannitol bathing solution during a 0.5 to 2.5 h period immediately after warming the tissue to 20°C. These data represent the means of three replicates.

<table>
<thead>
<tr>
<th>Conditioning Temperature</th>
<th>Holding Temperature</th>
<th>°C</th>
<th>% total conductivity h⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>12.5°C</td>
<td>2.5°C</td>
<td>42</td>
<td>0.6 a*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>37</td>
<td>0.7 a</td>
</tr>
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<td></td>
<td></td>
<td>25</td>
<td>0.9 a</td>
</tr>
<tr>
<td></td>
<td></td>
<td>12.5</td>
<td>0.9 a</td>
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</table>

* Means followed by the same letters are not significantly different at the 5% level by LSD test.
solution was significantly lower than from discs treated with water or no solution. Leakage from discs treated with 184 μM ABA was always lower than from the 0.16 and 1.6% ethanol controls after 6 d at 2.5°C, but the differences were not significant at the 5% level. After 6 d at 12.5°C, the 184 μM ABA treated discs had significantly higher rates of leakage than control discs treated with either level of ethanol. An ABA concentration of 1844 μM significantly decreased the rate of ion leakage from discs held at 2.5°C by over 50% compared to the controls, while increasing the rate of leakage from discs held at 12.5°C. Since these results indicated that ABA could play a role in reducing susceptibility of cucumber plants to chilling injury, the endogenous ABA content was determined in discs during temperature conditioning and subsequent holding at chilling and nonchilling temperatures.

Effect of Temperature Conditioning on Endogenous ABA Levels

The indirect ELISA recovered an average of 102 ± 5.9% (range of 96–109%) of authentic ABA (50 pg) that had been added to samples of crude cucumber cotyledon extract diluted 15-, 20-, 30-, and 40-fold. Crude extracts from cotyledon discs that had been exposed to 25°C for 6 h and that were diluted 20- or 40-fold did not interfere with the linearity of the immunoassay over the range of 15 to 100 pg added ABA. Further evidence for the validity of the assay came from the parallelism of the 20-fold dilution of extracts from discs exposed to 12.5 or 37°C for 6 h and spiked with from 0 to 70 pg ABA. These discs initially contained different levels of endogenous ABA.

Changes in endogenous ABA concentration with conditioning are summarized in Table III. The base level of ABA in the discs exposed for 6 h to the growth temperature of 25°C was 53 ng ABA g⁻¹ fresh weight. ABA levels in discs exposed for 6 h at 37°C were 47 ng ABA g⁻¹ fresh weight. Such levels were significantly lower at the 5% level than in discs exposed at 12.5°C (67 ng ABA g⁻¹ fresh weight). This pattern of lower ABA content in discs exposed at 37°C was still evident after 4 d of holding at 12.5 or 2.5°C. Although holding the discs for 4 d significantly increased the endogenous level of ABA at both holding temperatures, the increase was greater at 12.5 than at 2.5°C, but this distinction was only significant for discs conditioned at 12.5°C.

Temperature Conditioning and Induction of HSPs

The five most prominently induced proteins in the total homogenate from tissue exposed at 37 or 42°C for 6 h, compared to the 12.5 or 25°C controls, had apparent molecular mass of 25, 38, 52, 70, and 80 kD (Fig. 2, cf. lanes A and B with lanes C and D). The HSPs induced by 6 h at 37°C (Fig. 2, lane A) either disappeared or were significantly reduced in concentration by 4 d of holding at either 2.5 or 12.5°C (Fig. 2, lanes E and F).

The intensity of three other proteins with apparent molecular mass of 14, 17, and 43 kD were reduced by the high temperature treatments (Fig. 2, cf. lanes A and B with lanes C and D). The intensity of the 43 kD band recovered during 4 d of holding at either 12.5 or 2.5°C, but the other bands showed only slight changes upon holding at either temperature (Fig 2, cf. lane A with lanes E and F).

DISCUSSION

Chilling Injury and Temperature Conditioning

Ion leakage, used as a gross measurement of membrane permeability, increased from discs of cucumber cotyledons after 2 d of chilling at 2.5°C in a water saturated atmosphere as compared to the rate of leakage from discs held at the nonchilling temperature of 12.5°C (Fig. 1). This result confirms that of Rikin and Richmond (20) who demonstrated that, in addition to the harmful effects of low temperatures on reducing the content of cell water in cucumber cotyledons, low temperatures by themselves can damage these chilling sensitive tissues.

The 6 h temperature conditioning treatments prior to chilling affected the extent of chill-induced injury (Fig. 1; Table III).
Figure 2. Effect of conditioning and holding temperatures on the incorporation of \(^{35}\text{S}\)amino acids into soluble protein. Discs from 11-d-old cucumber seedlings were subjected to the following conditioning temperatures for 6 h: 37°C (lanes A, E, and F); 42°C (lane B); 12.5°C (lanes C, G, and H); and 25°C (lane D). The discs were incubated with \(^{35}\text{S}\)amino acids for the last 4 h of the 6 h temperature treatment. Some discs were subsequently held at 12.5°C (lanes F and H) or at 2.5°C (lanes E and G) for 4 d after exposure. The same cpm were loaded in each lane. Molecular mass standards (in kD) are indicated to the left of lane A, and the five most prominent protein changes are indicated with an arrow.

I). The 6 h conditioning at 37 or 42°C significantly reduced chill-induced ion leakage, while the 6 h conditioning at 12.5 or 25°C treatment had no effect on protecting the plant against chilling. This high-temperature conditioning effect on chilling sensitivity has also been observed in our laboratory with whole tomato fruit and pericarp discs of tomato fruit, and with pericarp discs of cucumber fruit (data not shown). This response is also consistent with the beneficial effect of conditioning whole cucumber fruit for 24 h at 36 to 40°C on subsequent chilling injury reported by Hirose (9).

Some physiological changes have been associated with an analogous conditioning of sensitive tissue near the chilling temperature, a practice called hardening (23, 26). Among these changes are increases in the degree of unsaturation of fatty acids in the phospholipids of membranes in a number of plants (26). The increase in sugars and starch, and the decrease in RNA, protein, and lipid-soluble phosphate have been reported in cotton plants after hardening at 15°C (7).

Carbohydrate accumulation, following light exposure, increases the tolerance of tomato seedlings to chilling (11). In a series of preliminary experiments, we failed to detect a significant difference among the levels of soluble sugars in fully expanded cucumber cotyledons that were held in the dark for up to 4 d during conditioning. Although leaves and cotyledons are metabolically different structures, one being predominantly a source and the other being a sink, our results indicate that the beneficial effect of high-temperature conditioning on chilling injury (Fig. 1) is unlikely due to carbohydrate accumulation.

ABA and Chilling Injury

Markhart (17) reported that ABA-induced changes in root hydraulic conductance alleviated the chilling-induced water stress in soybean. Rikin and Richmond (20) found that ABA applications to whole cucumber seedlings 15 h before the cotyledons were detached and exposed to chilling temperatures, resulted in less ion leakage than from the nonsprayed, chilled control plants. The beneficial effect of exogenous ABA applications on reducing susceptibility to chilling was confirmed in our study by applying ABA to excised cotyledon discs (Table II). However, the level of ABA required to produce a significant reduction in chilling-induced ion leakage may have exceeded physiological levels. Such high external concentrations may be necessary to induce physiologically active levels in the tissue because of limited absorption. Rather than continue our study of the effect of externally applied ABA, we decided to measure endogenous levels of ABA in tissue that had been conditioned to have different chilling sensitivities.

Stressful temperatures, by themselves, have been shown to induce changes in endogenous ABA levels in both warm- and cool-season vegetable crops (5). No data on the effect of temperature on ABA levels in cucumber has been reported. An indirect ELISA for ABA was selected because it is more sensitive than a direct ELISA and considerably less time-consuming than the gas chromatography and HPLC methods. The sensitivity of the indirect ELISA using a commercial monoclonal antibody and the ABA-4'-BSA conjugate was as low as 5 pg ABA. Purification of the sample was not required for linearity of the assay as was shown by the parallelism of dilution curves for extracts containing different amounts of endogenous ABA and the almost 100% recovery of ABA from crude extracts.

Since water stress can increase ABA levels in plants (25), special attention was paid to minimize water loss at all temperatures selected in this study. Under the experimental conditions used, water loss was always lower than 2% and not significantly different among treatments. Thus, changes observed in ABA levels can be related to changes in temperature. ABA levels of tomato plants exposed to low and high-stressful diurnal temperatures of 10 to 15°C and 35 to 45°C, respectively, were significantly higher than those of plants grown at the optimal temperature of 25°C (5). After 72 h exposure to 25°C, the ABA levels were almost constant and lower than 150 ng g\(^{-1}\), whereas at the high- or low-stressful temperatures ABA ranged from 165 to 280 ng g\(^{-1}\). ABA levels changed with the temperature and duration of exposure, but the highest ABA levels were always observed in plants exposed to the coolest temperature examined (i.e. 10°C). When soil moisture was maintained at an adequate level to avoid both water stress and drought-induced chilling tolerance, the ABA levels in other warm-season crops such as bean, corn, eggplant, muskmelon, and okra after 48 h at 10°C ranged

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from 17 to 42 μg g⁻¹. These levels were considerably higher than in the same plants preconditioned at 40°C, and generally only slightly higher than in plants preconditioned at 25°C. In contrast, of the cool-season crops such as beet, cabbage, lettuce, and radish, only peas had significantly higher ABA levels after 48 h at 40°C than at 10 or 25°C (5).

Since the rate of ion leakage from discs of cucumber cotyledons that were conditioned at 12.5°C and chilled at 2.5°C for 4 d was considerably higher than from discs conditioned at 37°C and chilled (Table I), and the ABA levels were also significantly higher in those discs conditioned at 12.5°C than at 37°C (irrespective of the subsequent holding temperature) (Table III), we concluded that the reduction in susceptibility of discs to chilling injury by conditioning at 37°C was not due to temperature-induced increases in endogenous levels of ABA. The higher level of ABA in cucumber disc conditioned at 12.5°C than at 37°C may have occurred as the result of low-temperature hardening of this warm-season crop. However, the physiological levels of ABA induced were not high enough to reduce susceptibility to chilling.

**Heat Shock Proteins and Chilling**

Synthesis and accumulation of HSPs has been associated with the prevention of high temperature stress-induced cellular leakage (13). HSPs have been roughly divided into three groups ranging in size from 15 to 18 kD, from 20 to 33 kD, and from 68 to 104 kD (3, 10). In preliminary experiments, the induction of proteins in cucumber cotyledon discs with similar molecular mass to the two higher groups of HSPs occurred within 30 min of transfer to 37 and 42°C, but not upon transfer to 12.5 or 25°C (data not shown). After 6 h of exposure, five proteins with apparent molecular mass of 25, 38, 50, 70, and 80 kD were induced at 37 or 42°C in comparison to the 12.5 or 25°C controls (indicated by arrows in Fig. 2). Surprisingly, we were unable to detect proteins in the small 15 to 18 kD size range. Although we refer to each band as a protein, we realize that each band may be composed of a collection of polypeptides with similar molecular mass and that a two-dimensional gel could possibly resolve each band into a number of spots. However, each band does represent at least one protein.

Other proteins of molecular mass 14, 17, and 43 kD, that were expressed at 12.5 or 25°C, were markedly reduced by the thermal stress treatments. Synthesis of the 43 kD protein recovered upon subsequent holding at 12.5 or 25°C for 4 d, but synthesis of the 14 and 17 kD proteins showed little recovery even after the 4 d holding period. These protein changes agree with those occurring during HSPs induction. Heat shock causes alterations of gene expression in plants resulting in the induction of HSPs which appears to compensate for the suppression of the synthesis of some of the normally expressed proteins (10). Many preexisting mRNAs are sequestered rather than degraded (1), or can undergo normal turnover (14). The synthesis of normally expressed proteins resumes when plants are returned to nonstressed temperatures.

Changes in other proteins appeared to be the result of aging of the tissue during holding. Three proteins that had an apparent molecular mass of 41, 66, and 105 kD and had not been affected by the conditioning treatments increased in intensity during holding, with greater intensity related to conditioning and temperature treatments that would have induced the most rapid aging (i.e. 37°C/12.5°C > 12.5°C/25°C > 37°C/2.5°C > 12.5°C/2.5°C).

Water stress and wounding have been shown to induce HSPs in maize (8). Nevertheless, the HSPs induced in cucumber cotyledons under our experimental conditions can be exclusively related to the heat shock treatment since samples were held in a water-saturated atmosphere and no apparent HSPs were detected in the 25°C control tissue which experienced the same level of water loss as the other temperature treatments.

Although the function of most of the HSPs has not been elucidated, these stress-induced proteins may allow plants to make structural and biochemical adjustments that enable them to cope with stress conditions. One approach that has been used to investigate the potential role of HSPs is to specify the cellular location of these proteins, since they could then be related to the function of specific organelles. Recent works have shown the association of HSPs with membranes, and the potential role of HSPs in maintaining normal membrane-associated processes during heat stress (10). Disorganization of cellular membranes measured as cell ion leakage is considered a normal symptom of chilling and heat stress (13, 23, 26). Prior treatment with HSPs-inducing temperatures preserved the plasma membrane structure (16), diminished the rate of solute leakage, and gave rise to the appearance of a 15 kD HSP at the plasma membrane level (13).

Our data indicated that ion leakage was reduced by the 37 or 42°C heat shock treatment, and that such reduction was concomitant with the synthesis of several HSPs. Some of these proteins (i.e. HSP 70) have been associated with the plasma membrane in maize roots (4).

It is also possible that other products accumulated with the HSPs during heat treatment and are responsible for the thermal tolerance. Stress temperatures, with their attendant temperature-induced water stress, induce ABA (5, 6) and ABA induces the synthesis of HSPs (8). Nevertheless, there was no direct relationship between endogenous ABA content and the rate of ion leakage following temperature conditioning in this study. We conclude that increased tolerance to chilling injury induced in cucumber cotyledons by temperature conditioning is more likely related to the appearance of HSPs, which would protect plants against a further stress temperature such as chilling, than to the induction of higher endogenous levels of ABA.

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