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Short Communication

Concomitant Changes in High Temperature Tolerance and Heat-Shock Proteins in Desert Succulents[1]

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ABSTRACT

Raising the day/night air temperatures from 30°C/20°C to 50°C/40°C increases the high temperature tolerated by *Agave deserti*, *Carnegiea gigantea*, and *Ferocactus acanthodes* by 6°C to 8°C; the increase is about half completed in 3 days and fully completed in 10 days. A 25 to 27 kilodalton protein concomitantly accumulates for all three desert succulents upon transfer to 50°C/40°C, while accumulation of other heat "heat-shock" proteins is species specific. Some of the induced proteins are more abundant at 3 days, while others (including the 25–27 kilodalton protein) remain after completion of high temperature acclimation.

Tissue temperatures of desert succulents in their native habitats can appreciably surpass ambient air temperatures (6, 20). Cacti and agaves are apparently the most tolerant of high temperatures among higher vascular plants, surviving tissue temperatures in excess of 60°C (5, 19, 21). Moreover, the high temperatures tolerated by these succulents increase in response to increasing growth temperatures. For *F. acanthodes* the high temperature tolerated is correlated with the fatty acid saturation level, but this is not the case for *A. deserti* and *C. gigantea* (7). Hence, some other factor must be responsible for the changes in the high temperatures tolerated by these desert succulents.

Recently, various tissues have been shown to synthesize "heat-shock" proteins in response to a high temperature treatment, and these HSPs[2] have been proposed to afford protection against otherwise lethal temperatures (4, 8, 10–15, 17). Kinetics of HSP synthesis are well correlated with increases in the high temperature tolerated by mammalian cells (11, 12). The protein synthesis inhibitor cycloheximide can prevent an increase in the high temperature tolerated (14, 17), while arsenite can induce HSP synthesis and thus provide high temperature protection without any prior heat shock (13). In addition, mutants impaired in synthesizing HSPs show no increase in the high temperature tolerated in response to heat treatment (15, 17). However, the need for HSPs to maintain an acquired high temperature tolerance has not been fully demonstrated, because HSP synthesis need not imply HSP accumulation.

Attempts to demonstrate HSP accumulation in maize led to the conclusion that HSPs do not significantly accumulate (2, 3). In fact, even HSP synthesis can be transient under continuous heat-shock temperatures (2, 3, 18). Nevertheless, incorporation of ^{35}S into a 100 kD HSP in yeast was correlated with the high temperature tolerated (17), and water and high temperature stressed cotton plants accumulated at least 8 proteins not accumulated in control plants (1). If accumulation of HSPs is necessary for the acquisition and maintenance of increased high temperature tolerance, then such a phenomenon should be evident in desert succulents upon increasing growth temperatures. Here, three succulent species native to the hot Sonoran Desert of North America, *A. deserti*, *C. gigantea*, and *F. acanthodes*, were shifted from moderate day/night air temperatures of 30°C/20°C to high temperatures of 50°C/40°C. Changes in the protein size patterns of the chlorenchyma tissue were monitored for times that led to marked increases in the high temperatures tolerated by this tissue.

MATERIALS AND METHODS

Plant Material. *Agave deserti* (Engelm.) and *Ferocactus acanthodes* (Lem.) Britton and Rose were collected from the Philip

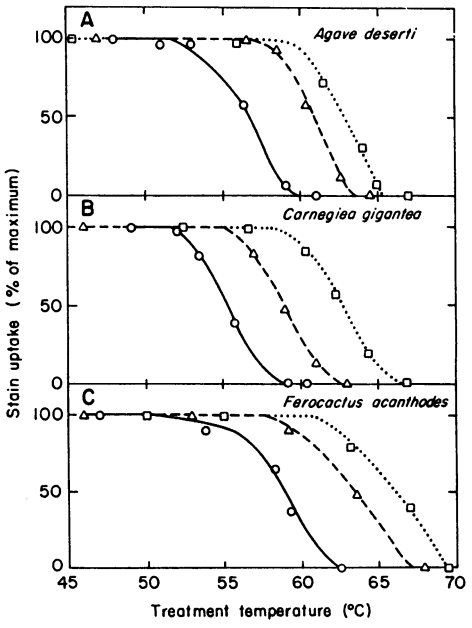

FIG. 1. Influence of a 1-h high temperature treatment on uptake of a vital stain for three species of desert succulents maintained for at least 30 d under day/night air temperatures of 30°C/20°C (○), shifted to 50°C/40°C for 3 d (△), or kept at 50°C/40°C for 20 d (□).

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[2] Abbreviation: HSPs, heat-shock proteins.

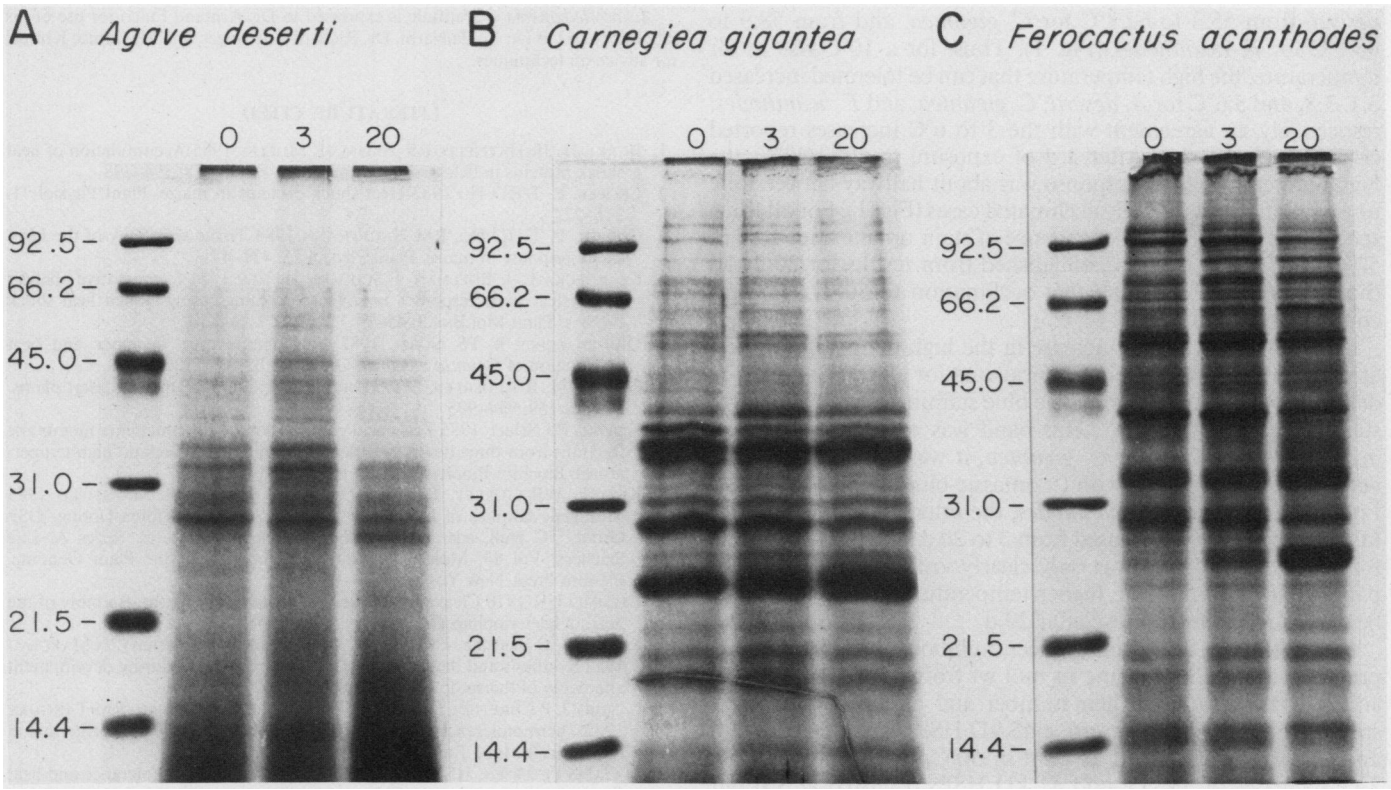

FIG. 2. Influence of increasing growth temperatures on the chlorenchyma proteins of three species of desert succulents. At specific times (indicated in days above each lane) following the shift in the day/night air temperatures from 30°C/20°C to 50°C/40°C, proteins were extracted from the chlorenchyma, electrophoresed in SDS-polyacrylamide gels, and stained with Coomassie blue. Numbers in the left margins indicate mol wt (in kD) of protein standards.

L. Boyd Deep Canyon Desert Research Center, 8 km south of Palm Desert, California (at 33°38′N, 116°24′W, 850 m elevation). *Carnegiea gigantea* (Engelm.) Britton and Rose was collected from a site 15 km west of Wittman, Arizona (at 33°46′N, 112°41′W, 500 m). Plants were maintained at 30°C/20°C day/night air temperatures in an M-31 Environmental Growth Chamber as previously described (7). High temperature treatments were accomplished by shifting the plants to 50°C/40°C day/night air temperatures (watering frequency was increased to at least twice weekly to compensate for greater water loss at the elevated temperatures).

High Temperature Tolerance. Pieces of mature shoot tissue were immersed in water baths maintained over a range of temperatures (each ± 0.2°C) for 1 h before transfer to 4°C for 1 d to improve stain uptake (5, 19). Fresh sections about 2 cell layers thick were taken from the chlorenchyma and stained with 0.3 mM neutral red (3-amino-7-dimethylamino-2-methylphenazine [HCl]) in 7 mM KH_2PO_4/Na_3PO_4 at pH 7.4 (5). Approximately 500 cells were examined microscopically for stain uptake into the vacuole, a measure of membrane integrity and cell viability (5, 21). Stain uptake is indicated relative to a treatment temperature of 30°C; tissue death occurred at temperatures that reduced stain uptake to zero.

Protein Extraction. Mature chlorenchyma tissue (1–2 g) was excised with a razor blade and ground in a mortar containing washed sand, 25 ml of 50 mM Tris-HCl (pH 7.0), 2% (w/v) SDS, 0.05% (w/v) sodium mercaptobenzothiazole, 2.5 mM *p*-hydromercuribenzoate, 1 mM phenylmethylsulfonylfluoride (added just before homogenization from a 100 mM solution in DMSO), and 1 drop of Antifoam C Emulsion (Sigma). Coarse debris was removed by filtration followed by centrifugation at 3000*g* for 15 min. Five volumes of cold acetone (at −20°C) were added to the decanted supernatant and the mixture stored overnight at −20°C. The resulting precipitate was collected by centrifugation (12,000*g* for 5 min), the pellet washed once with cold acetone, and the final pellet dried under vacuum before pulverizing and storing at −70°C. Protein content of the powder was determined by the method of Lowry *et al.* (16) using *A* at 660 nm; defatted BSA was used as the protein standard.

Gel Electrophoresis. Proteins were analyzed using Laemmli's one-dimensional SDS-PAGE (9). The proteinaceous powder was dissolved in 6 mM Tris-HCl (pH 6.8), 2% (w/v) SDS, 10% (v/v) glycerol, 5% 2-mercaptoethanol, and 0.001% (w/v) bromophenol blue. After heating to 100°C for 2 min and then centrifuging at 1000*g* for 5 min, the supernatant was placed onto a 4% (w/v) polyacrylamide stacking gel on top of a 12% (w/v) polyacrylamide separating gel 12 cm long and 0.75 mm thick. Electrophoresis was at 50 V. Proteins were made visible with either Coomassie blue or silver stain (Bio-Rad Silver Stain Kit; Bio-Rad, Richmond, CA). Staining in 0.1% (w/v) Coomassie blue, 50% (v/v) methanol, and 7% (v/v) glacial acetic acid was for 30 min; unbound Coomassie blue was removed with 7% (v/v) glacial acetic acid. For mol wt determinations the proteins were coelectrophoresed with the following Bio-Rad standards: lysozyme (14.4 kD), soybean trypsin inhibitor (21.5 kD), carbonic anhydrase (31.0 kD), ovalbumin (45.0 kD), BSA (66.2 kD), and phosphorylase *b* (92.5 kD).

RESULTS AND DISCUSSION

All three species of desert succulents responded to a shift in day/night air temperatures from 30°C/20°C to 50°C/40°C by increases in the high temperatures tolerated by their tissues. After a 20-d exposure, the high temperature at which 50% inhibition of stain uptake occurred increased from 56.7 to 62.9°C for *A.*

deserti, from 55.3 to 62.8°C for *C. gigantea*, and from 58.9 to 66.1°C for *F. acanthodes* (Fig. 1). Thus, for a 10°C rise in air temperature, the high temperature that can be tolerated increased 3.1, 3.8, and 3.6°C for *A. deserti*, *C. gigantea*, and *F. acanthodes*, respectively, in agreement with the 3 to 6°C increases reported elsewhere (5, 19, 21). After 3 d of exposure to 50°C/40°C, the high temperature stain response was about halfway between the unacclimated and the fully acclimated cases (Fig. 1). For all three species the temperature dependence of stain uptake after 10 d at 50°C/40°C could not be distinguished from results for 20 or 30 d at 50°C/40°C, indicating that acclimation to 50°C/40°C was complete in about 10 d.

Concomitant with the increase in the high temperature tolerated, a 25 to 27 kD protein accumulated for all three species, as demonstrated by both Coomassie blue staining (Fig. 2) and silver staining (data not shown). This band was most evident for *F. acanthodes* (Fig. 2C); for *C. gigantea*, it was more readily seen with silver staining than with Coomassie blue staining (Fig. 2B). For *C. gigantea* and *F. acanthodes*, the intensity of the 25 to 27 kD band noticeably increased from 3 to 20 d at 50°C/40°C, while for *A. deserti* the band was only clearly visible at 20 d. Protein patterns after 10 d at the higher temperature regime could not be distinguished from those after 20 d.

In addition to the common accumulation of a 25 to 27 kD protein, other HSPs ranging in mol wt from 15 to 104 kD also accumulated, although their number and amount was species specific (Fig. 2). For *A. deserti*, a 45 kD HSP was detected at 3 d and increased in amount at 20 d of 50°C/40°C exposure (Fig. 2A); increases of 33, 35, and 81 kD HSPs occurred at 3 d but they decreased at 20 d (Fig. 2A). For *C. gigantea*, a 104 kD HSP accumulated at both 3 and 20 d, while increases in 41, 56, and 62 kD HSPs were observed at 3 d only (Fig. 2B). For *F. acanthodes*, a transient increase in a 54 kD HSP was detected, accumulation of 15 and 22 kD HSPs occurred at 3 and 20 d, and increases of 62 and 73 kD HSPs occurred when acclimation was complete at 20 d (Fig. 2C).

There appear to be two sets of HSPs, one that is maintained at elevated levels even after development of high temperature tolerance is complete, and another that only occurs during the acclimation process. The transient nature of the latter set is in concordance with the transient nature of HSP synthesis in maize (2, 3) and yeast (18). Although the significance of the two sets of HSPs remain unclear, one set may be involved with the acquisition process while the other set may be needed to maintain the high temperature tolerance.

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