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A Class of Soybean Low Molecular Weight Heat Shock Proteins[1]

Immunological Study and Quantitation

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ABSTRACT

Two major polypeptides of the 15- to 18-kilodalton class of soybean (*Glycine max*) heat shock proteins (HSPs), obtained from an HSP-enriched $(NH_4)_2SO_4$ fraction separated by two-dimensional polyacrylamide gel electrophoresis, were used individually as antigens to prepare antibodies. Each of these antibody preparations reacted with its antigen and cross-reacted with 12 other 15- to 18-kilodalton HSPs. With these antibodies, the accumulation of the 15- to 18-kilodalton HSPs under various heat shock (HS) conditions was quantified. The 15- to 18-kilodalton HSPs began to be detectable at 35°C, and after 4 hours at 40°C they had accumulated to a maximum level of 1.54 micrograms per 100 micrograms of total protein in soybean seedlings and remained almost unchanged up to 24 hours after HS. Accumulation of the HSPs was reduced at temperatures higher than 40°C. At 42.5°C the HSPs were reduced to 1.02 micrograms per 100 micrograms, and at 45°C they were hardly detectable. A brief HS at 45°C (10 minutes), followed by incubation at 28°C, which also induced HSP synthesis, resulted in synthesis of this class of HSPs at levels up to 1.06 micrograms per 100 micrograms of total protein. Taking into consideration the previous data concerning the acquisition of thermotolerance in soybean seedlings, our estimation indicates that the accumulation of the 15- to 18-kilodalton HSPs to 0.76 to 0.98% of total protein correlated well with the establishment of thermotolerance. Of course, other HSPs, in addition to this group of proteins, may be required for the developement of thermotolerance.

The induction of HSP[2] synthesis in response to thermal stress occurs in all organisms examined, ranging from bacteria to humans (20). Although the physiological function of HSPs has not been clearly documented, their induction is well correlated with thermotolerance in a time- and temperature-dependent manner. On the basis of this correlation, it has been hypothesized that accumulation of HSPs is an essential component for protection from heat damage (10, 12, 16, 19). Although studies of *Drosophila* have provided a basis for research in many systems, there are significant differences among organisms in response to HS. A major difference is the relative abundance of proteins synthesized during HS. In *Drosophila*, the major HSPs are 83, 70, 68, 27, 26, 23, and 22 kD, and have a predominant synthesis of the 70-kD HSP (13). Although plants synthesize HSPs with molecular masses of 92, 84, 70, 68, and 15 to 27 kD, the complex group of 15- to 27-kD proteins predominates (7, 23).

We have been studying the 15- to 18-kD class of HSPs, which are synthesized when the seedlings are shifted from a normal growth temperature of 28 to 40°C. These 15- to 18-kD HSPs accumulate to a detectable level, as seen by SDS-PAGE separation followed by Coomassie blue staining (15). They are present in the cytosol and become associated with organelles in response to thermal stress (12).

Recently, we showed that isolated mitochondria that had this 15- to 18-kD class of HSPs associated with them were functional in oxidative phosphorylation at an HS temperature of 42.5°C, suggesting that this class of HSPs protected these organelles from heat damage (1). Furthermore, we demonstrated that the protection of soluble proteins from heat denaturation *in vitro* occurred with the addition of an HSP-enriched aliquot that was predominantly composed of the 15- to 18-kD class HSPs (4).

In this study of the 15- to 18-kD class of HSPs in soybean seedlings, we show that 13 polypeptides, as resolved by 2-D PAGE, are immunologically related. We establish a quantitative immunological assay for the 15- to 18-kD HSPs under various HS conditions. In addition, our results indicated that HS conditions known to confer thermotolerance are associated with accumulation of a significant level of the 15- to 18-kD HSPs. These results further support the notion that these HSPs play a role in thermotolerance.

MATERIALS AND METHODS

Plant Material

Soybean seeds (*Glycine max* cv Taita Kaoshiung No. 8) were germinated in rolls of moist paper towels at 28°C in a dark growth chamber as described by Lin *et al.* (12).

Preparation of HSP-Enriched Protein Fraction

Two-day-old soybean seedlings (3–4 cm in length) without cotyledons were incubated in a medium containing 5 mM

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[2] Abbreviations: HSPs, heat shock proteins; HS, heat shock; LMW, low molecular weight; 2-D, 2-dimensional; HRP, horseradish peroxidase; PRS, postribosomal supernatant; TBS, 20 mM Tris-HCl(pH 7.5), 0.5 M NaCl; TTBS, TBS plus 0.05% Tween 20; AU, absorbance units.

potassium phosphate (pH 6.0) and 1% sucrose before treatment at different temperatures for varying periods of time. Seedlings were harvested and homogenized with a Polytron equipped with a PT-20 probe in a medium containing 0.5 M sucrose, 0.25 M Tris-HCl (pH 8.8), 30 mM $MgCl_2$, 0.1 M KCl, 1 mM DTT, and 1 mM PMSF. The homogenate was filtered through a layer of Miracloth, and the filtrate was further centrifuged at 300,000*g* for 2 h, as described previously (4) for the preparation of the PRS. The PRS was further fractionated by precipitation with $(NH_4)_2SO_4$ at 65% to 100% saturation. The precipitate was dissolved in buffer containing 50 mM Tris-HCl (pH 8.0), 1 mM EDTA, and 0.1% 2-mercaptoethanol before overnight dialysis against three changes of the same buffer. All experimental procedures were carried out at 4°C.

2-D Electrophoresis and Antibody Preparation

For separation of proteins by 2-D PAGE, the dialyzed 65 to 100% $(NH_4)_2SO_4$ fraction was added to four volumes of chilled acetone (-20°C) and allowed to stand overnight to precipitate proteins. The precipitate was pelleted, dried, and resuspended in O'Farrell sample buffer (18). Proteins were resolved by isoelectric focusing in the first dimension and SDS-PAGE with a 15% (w/v) gel in the second dimension.

Coomassie blue R-250 was used to visualize the protein spots on the gels. For preparation of antibodies, two major HSP spots, identified by Coomassie blue staining were cut out of the gel separately and washed thoroughly with distilled water. About 35 μg of each HSP was collected from 50 2-D gels, homogenized with PBS, emulsified with complete Freund's adjuvant, and injected subcutaneously into several sites on the back of an adult New Zealand White rabbit. The animal was boosted two times at 10- to 14-d intervals in the same manner, except that incomplete Freund's adjuvant was used and the amount injected was reduced to half of the initial concentration. Two weeks after the final injection, 25 mL of blood was drawn from the ear vein and allowed to clot at room temperature for 1 h and then left overnight at 4°C. The serum was collected, $(NH_4)_2SO_4$ was added to 45% saturation, and the precipitate was collected by centrifugation. The pellet was washed twice with 45% $(NH_4)_2SO_4$. The precipitate was dissolved in a buffer containing 50 mM Tris-HCl (pH 8.6), 0.15 M NaCl and dialyzed overnight against three changes of the same buffer. After dialysis, the serum sample was brought up to the same volume as the collected serum with the same buffer and stored at -20°C.

SDS-PAGE and Immunoblotting

Total proteins were extracted from soybean seedlings in 5 mL of SDS extraction buffer as described previously (12). The protein content of the final sample was determined by the procedure of Lowry (14). One-dimensional gel electrophoresis was performed according to Laemmli (9) with 15% (w/v) gels. For 2-D PAGE, the method of O'Farrell was used (18). After electrophoresis, the gel was immersed in the transfer buffer (25 mM Tris, 192 mM glycine [pH 8.3], 20% [v/v] methanol) for 30 min before blotting. Protein transfer was performed with a TE-52 transfer unit (Hoefer, San Francisco, CA) according to the method of Towbin *et al.* (22). Proteins were electroblotted onto 0.45-μm Immobilon PVDF transfer membranes (Millipore) for 3 to 4 h at 0.7 A. Buffers were used only once, and the temperature was maintained at 4°C during transfer. After transfer, the blots were blocked with 3% gelatin TBS. The gelatin used in this blocking step was dissolved at 37°C and allowed to cool to room temperature during a 1-h incubation. Blots were then incubated in primary antibody at 1:50 dilution with gentle agitation overnight at room temperature. Antibody solution was freshly prepared in TBS containing 1% gelatin. After washing three times in TTBS for 10 min per wash, the blots were incubated with a 1:2500 dilution of HRP-conjugated goat anti-rabbit IgG (Bio-Rad) in TTBS containing 1% gelatin for 1 h at room temperature. Blots were then transferred to clean trays and exposed to HRP color development reagent containing 4-chloro-1-naphthol (Bio-Rad) to develop the colored reaction product. The color reaction was allowed to develop for a few minutes and was stopped by rinsing in distilled water under the conditions outlined in the instructions that accompany the assay kit (Bio-Rad).

Quantitative Estimation of HSPs

The members of the 15- to 18-kD class of HSPs that cross-reacted with the antibody (13 spots) were cut from 2-D gels, bulked, and eluted, and the amount of protein was estimated with Lowry's method (14). A known amount of these proteins was then run on SDS-PAGE and blotted onto Immobilon PVDF transfer membrane, and the color was developed after reaction with HSP antibody and a second, anti-rabbit antiserum conjugated with HRP. The color that developed was scanned with an LKB laser densitometer (Gel Scan XL, Software 2-D Program Version 2.0, LKB). The color values were expressed as AU × mm^2, according to the manual.

RESULTS

Preparation of LMW HSP Antibodies

When soybean seedlings were shifted from 28°C to HS temperatures (40°C), HSP synthesis was induced. These HSPs were enriched in the 65% to 100% $(NH_4)_2SO_4$ fraction prepared from the PRS (4). This $(NH_4)_2SO_4$ fraction was resolved by 2-D PAGE and stained with Coomassie blue. The 15- to 18-kD HSPs accumulated to a stainable level after HS treatment (Fig. 1A). Thick arrows with asterisks in Figure 1A indicate the polypeptides that were cut out individually from the 2-D gels, collected, and used separately as antigens for preparation of monospecific antibodies. The antibodies prepared from either polypeptide cross-reacted with at least 13 polypeptides of the 15- to 18-kD LMW HSP group (Fig. 1B). Neither high mol wt HSPs nor LMW HSPs in the range of 20,000 to 30,000 were found to cross-react with the antibodies. This indicates that the antigenicity of 15- to 18-kD HSPs is different from that of the other families of HSPs.

Development of a Quantitative Assay for LMW HSPs

The 13 eluted polypeptides that cross-reacted with the antibodies on 2-D gels were used as a protein source for

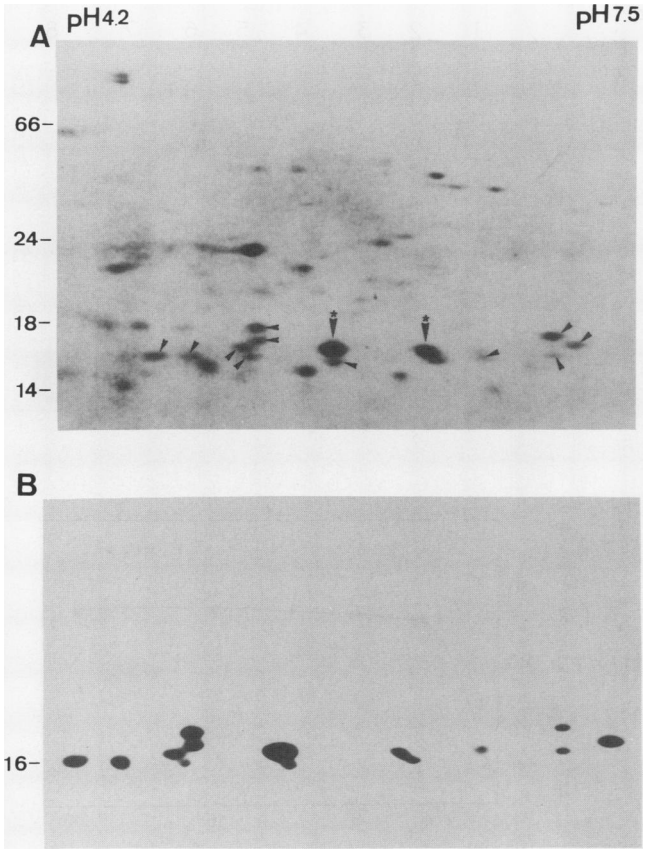

Figure 1. Coomassie blue staining (A) and western blot analysis (B) of proteins from 40°C heat-shocked seedlings after 2-D PAGE. LMW HSPs indicated with a large thick arrow and an asterisk were used individually for antiserum preparation. Cross-reacting polypeptides in panel B are equivalent to the Coomassie blue-stained spots indicated by arrows in panel A.

standardization in a quantitative assay. Known amounts of the eluted preparation were separated on SDS-PAGE for immunoblot analysis (Fig. 2A). The intensity of the reaction was determined with an LKB densitometer and the soft 2-D program and is expressed as AU × mm^2. A linear relationship between the color unit (AU × mm^2) and the amount of protein applied was obtained, and the assay technique was sensitive enough to detect as little as 0.5 μg of HSP (Fig. 2B).

To determine the optimum protein concentration for quantitation of HSPs from whole seedlings, soybean seedlings were heat shocked for 3 h at 40°C, and samples with crude protein content ranging from 12.5 to 100 μg were applied to a gel for immunoblot analysis (Fig. 3A). Quantitation of the result indicates that the amount of 15- to 18-kD HSPs detected is almost linear up to 100 μg of protein applied (Fig. 3B). Accordingly, a 50-μg sample of this total protein extract, which was in the range of linearity, was used as an internal standard in the subsequent quantitation experiments. When compared with the standard curve from Figure 2, the results obtained from Figure 3 indicate that the soybean seedlings treated for 3 h at 40°C contain 1.20 μg of 15- to 18-kD HSPs per 100 μg of total protein.

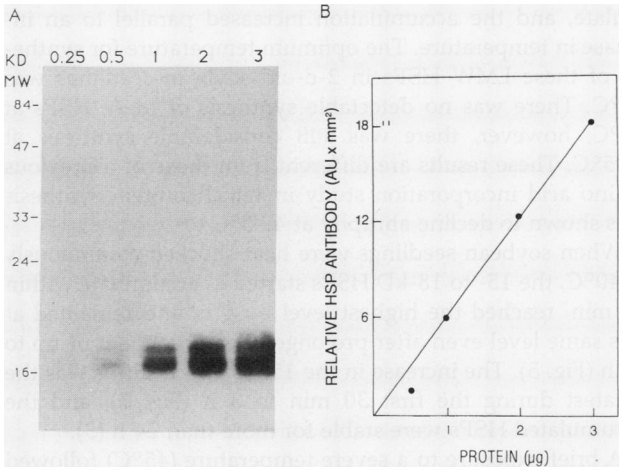

Figure 2. Standard curve for quantitative estimation of the LMW HSPs of 15- to 18-kD on western blots. All of the 15- to 18-kD HSPs shown in Figure 1 were collected from the gels, and a known amount of these HSPs was electrophoresed on a 1-D SDS gel. The gel was blotted to a nitrocellulose filter. The signal was generated by assaying the HRP conjugated to the second antibody (A). The developed western blots were scanned with an LKB laser densitometer with the 2-D version of the program. The results were plotted against the amount of protein applied to the gel (B).

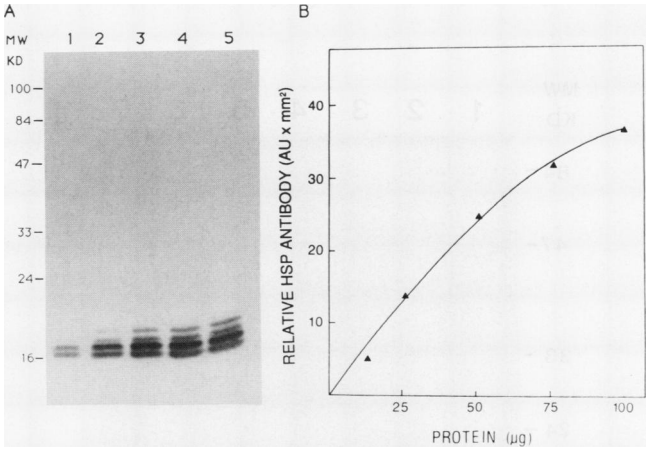

Figure 3. Linear range of the HSPs assayed from the total proteins extracted from soybean seedlings treated at 40°C for 3 h. The protein samples (12.5 μg [1], 25 μg [2], 50 μg [3], 75 μg [4], and 100 μg [5]) were separated by 1-D SDS-PAGE, blotted to nitrocellulose, reacted with the antibody and the second antibody conjugated to HRP, and assayed for HRP activity by color development (A). The relationship between the color developed (AU × mm^2) and the amount of protein applied is shown in panel B.

Accumulation of LMW HSPs under Various HS Conditions

Figure 4 shows the western blot analysis of the 15- to 18-kD HSPs accumulated during a 4-h period at different HS treatments. At 35°C, the 15- to 18-kD HSPs started to accumulate, and the accumulation increased parallel to an increase in temperature. The optimum temperature for synthesis of these LMW HSPs in 2-d-old soybean seedlings was 40°C. There was no detectable synthesis of these HSPs at 45°C; however, there was still considerable synthesis at 42.5°C. These results are different from those of a previous amino acid incorporation study in which protein synthesis was shown to decline abruptly at 42.5°C (6).

When soybean seedlings were heat shocked continuously at 40°C, the 15- to 18-kD HSPs started to accumulate within 30 min, reached the highest level by 4 h, and remained at this same level even after prolonged heat treatment of up to 24 h (Fig. 5). The increase in the 15- to 18-kD HSPs was the greatest during the first 30 min to 3 h (Fig. 5), and the accumulated HSPs were stable for more than 24 h (5).

A brief exposure to a severe temperature (45°C) followed by an incubation at the normal growth temperature also has been shown to induce a typical HS response (12). When the 15- to 18-kD HSPs induced by the 45°C pulse treatment were compared with HSPs synthesized at 40°C HS with a 2-D immunoblot analysis, it was found that the two treatments induced the same polypeptides (data not shown).

The amounts of 15- to 18-kD HSPs synthesized under the various HS regimes are summarized in Table I. When the seedlings were preheated at 40°C for 2 h, or at 28°C for 2 to 3 h after a brief HS at 45°C, the seedlings were shown to acquire thermotolerance (12). We estimated that under these treatments, the soybean seedlings synthesized and accumulated 0.76 to 0.98 μg of the 15- to 18-kD HSPs per 100 μg of total protein. Therefore, this level of the 15- to 18-kD HSPs appeared to be sufficient for the development of thermotolerance. Of course, a requirement of additional proteins besides these HSPs for acquiring thermotolerance may not be excluded.

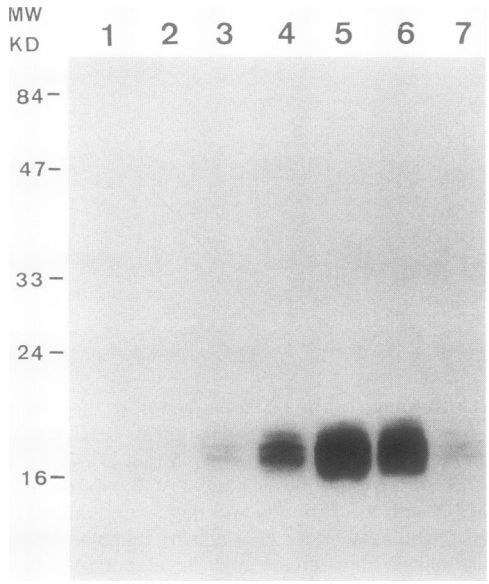

Figure 4. Western blot analysis of the LMW HSPs synthesized at different HS temperatures for 4 h. Protein samples (50 μg) from seedlings treated at 28°C (1), 32.5°C (2), 35°C(3), 37.5°C (4), 40°C (5), 42.5°C (6), and 45°C (7) were analyzed by SDS-PAGE.

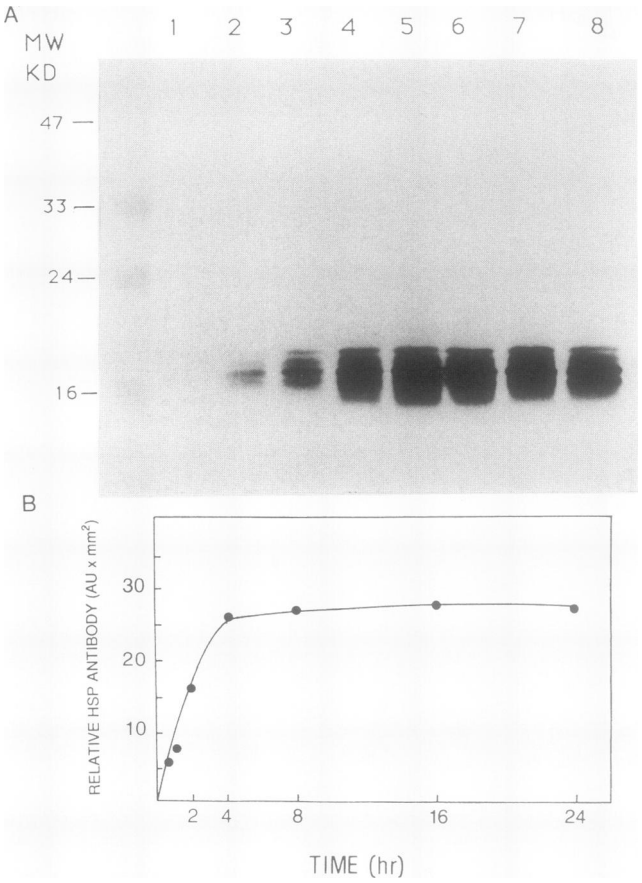

Figure 5. Western blot analysis of the 15- to 18-kD HSPs synthesized under continuous HS at 40°C. Protein samples (50 μg) from seedlings treated at 28°C (1) or at 40°C for 0.5 h (2), 1 h (3), 2 h (4), 4 h (5), 8 h (6), 16 h (7), and 24 h (8) were analyzed by 1-D PAGE (A). The signal was generated as described above. Results were plotted against the time of the HS treatment (B).

DISCUSSION

We were able to make antibodies using the most densely stained LMW HSPs with a molecular mass of 17.5-kD and a pI of either 6.1 or 6.3 (shown in Fig. 1B) as antigens. Each of these two antibody preparations showed immunological cross-reactivity with itself and 12 other polypeptides of the 15- to 18-kD class of HSPs. This immunological property allowed us to do quantitative studies of the 15- to 18-kD class of HSPs. Schöffl and Key (21) found that mRNA that has been hybrid-selected by several HS-specific cDNA clones was translated into 13 polypeptides of the 15- to 18-kD class of HSPs, and Nagao *et al.* (17) isolated four genes for this class of HSPs that had more than 90% homology in derived amino acid sequence. These results, along with the immu-

Table I. *Quantitative Estimation of 15- to 18-kD HSPs Synthesized under Various HS Regimens*

Each value is the mean ± SE of three separate experiments.

	HS Treatments		HSP Synthesized
	Temperature	Time	
	°C	h	μg HSPs/100 μg of protein
1.	32.5	4.0	0
	35.0	4.0	0.16 ± 0.01
	37.5	4.0	0.66 ± 0.03
	40.0	4.0	1.54 ± 0.27
	42.5	4.0	1.04 ± 0.09
	45.0	4.0	0
2.	40	0.5	0.38 ± 0.04
		1.0	0.60 ± 0.06
		2.0	0.98 ± 0.13
		4.0	1.54 ± 0.29
		8.0	1.58 ± 0.08
		16.0	1.64 ± 0.05
3.	45 (10 min), 28	1.0	0.44 ± 0.05
		2.0	0.76 ± 0.01
		3.0	0.94 ± 0.08
		4.0	1.06 ± 0.11

nological cross-reactivity among this class of HSPs, suggest that all 13 HSPs are probably closely related, although sequence analyses of all of the HSPs in this class have not been completed.

Our previous findings indicated that the acquisition of thermotolerance was strongly correlated with the accumulation of HSPs (12). Members of the 15- to 18-kD class of HSPs behaved as a group in that they localized to organelles during the HS and relocated to the cytoplasm during recovery from HS (12). Therefore, it has often been suggested that this class of HSPs provides protection from thermal damage (12). We demonstrated that association of this class of HSPs with isolated mitochondria actually provided protection of the oxidative phosphorylation *in vitro* at high HS temperatures (1). In addition, the 15- to 18-kD HSP-enriched fraction was shown to stabilize other proteins from heat denaturation *in vitro* (4). Thus, it has been of great interest to determine the actual amounts of this class of HSPs that accumulate under various HS conditions.

The quantitative assay technique is very sensitive. It allowed us to estimate with reasonable accuracy quantities of HSPs as little as 0.5 μg applied to a 1-D gel. Our estimation of the amount of HSPs that accumulated at various temperatures agreed with and was almost parallel to results of previous studies of protein synthesis with labeled amino acids at different HS temperatures (6). One exception occurred at an HS of 42.5°C; although there was a precipitable drop in amino acid incorporation at this temperature, we still detected an accumulation of 1.04 μg of HSPs per 100 μg of total protein, which still accounted for two-thirds of the HSPs synthesized at 40°C. The discrepancy was probably due to inhibition of uptake of the labeled amino acid caused by cellular leakage at 42.5°C (11). In general, the accumulation of HSPs agreed with the mRNA levels reported by Kimpel *et al.* (8). Recently, DeRocher *et al.* (2) reported a positive correlation between accumulation of an 18.1-kD LMW HSP and the corresponding level of mRNA, which was in agreement with that reported by Kimpel *et al.* (8).

The estimated accumulation of the 15- to 18-kD class of HSPs reached a maximum level by 4 h at 40°C HS (1.54 mg/100 mg protein) and remained fairly constant (1.64 mg/100 mg protein) throughout the 24-h HS period. Previous studies indicated that the accumulated HSPs were stable for more than 24 h (5), whereas mRNAs for this class of HSPs were shown to decrease after 6 h of HS at this temperature (8). These observations support the notion that the unchanged level of HSPs during continuous HS was attributable to the stability of HSPs rather than to an established equilibrium between continuing synthesis and degradation. As postulated for the 70-kD HSPs in *Drosophila* system (13), a shutoff of transcription by autoregulation may also operate for this class of HSPs in this system. In a previous study, synthesis of HSPs at 40°C for 2 h or after a brief HS at 45°C for 10 min following incubation at 28°C for 2 h was shown to be sufficient to provide thermoprotection in soybean seedlings (12).

By our estimate in this study, the 15- to 18-kD class of HSPs accumulated to the level of 0.76 to 0.98 μg/100 μg protein in 2-d-old seedlings under these HS conditions. We conclude that accumulation to this level of this 15- to 18-kD class of HSPs is sufficient for soybean seedlings to survive at an otherwise lethal temperature, but we recognize that other classes of HSPs may not be excluded as contributing to the acquisition of thermotolerance. Whether members of the 15- to 18-kD class of HSPs act as chaperones, as has been proposed for the 90-, 70-, and 60-kD HSPs (3), or serves a strictly structural role needs further investigation.

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LITERATURE CITED

1. **Chou M, Chen YM, Lin CY** (1989) Thermotolerence of isolated mitochondria associated with heat shock proteins. Plant Physiol **89:** 617–621
2. **DeRocher AE, Helm KW, Lauzon LM, Vierling E** (1991) Expression of a conserved family of cytoplasmic low molecular weight heat shock proteins during heat stress and recovery. Plant Physiol **96:** 1038–1047
3. **Hemmingsen SM, Woolford C, van der Vies SM, Tilly K, Dennsis DT** (1988) Homologous plant and bacteria proteins chaperone oligomeric protein assembly. Nature **333:** 330–333
4. **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
5. **Key JL, Kimpel JA, Vierling E, Lin CY, Nagao RT, Czarnecka E, Schoffl F** (1985) Physiological and molecular analysis of the heat shock response inplants. *In* BG Atkinson, DB Walden, eds, Changes in Eukaryotic Gene Expression in Response to Environmental Stress. Academic Press, New York, pp 327–348
6. **Key JL, Lin CY, Ceglarz E, Schoffl F** (1982) The heat shock response in plants: physiological considerations. *In* ML Schlesinger, M Ashburner, A Tissieres, eds, Heat Shock from Bacteria to Man. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, pp 329–335.

7. **Kimpel JA, Key JL** (1985) Heat shock in plants. Trends Biochem Sci **10:** 353–357
8. **Kimpel JA, Nagao RT, Goekjian V, Key JL** (1990) Regulation of the heat shock response in soybean seedlings. Plant Physiol **94:** 988–995
9. **Laemmli UK** (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature **227:** 680–685
10. **Li GC, Werb Z** (1982) Correlation between synthesis of heat shock proteins and development of thermotolerence in chinese hamster fibroblast. Proc Natl Acad Sci USA **79:** 3218–3222
11. **Lin CY, Chen YM, Key JL** (1985) Solute leakage in soybean seedlings under various heat shock regimes. Plant Cell Physiol **26:** 1493–1498
12. **Lin CY, Roberts JK, Key JL** (1984) Acquisition of thermotolerence in soybean seedlings: synthesis and accumulation of heat shock proteins and their cellular localization. Plant Physiol **74:** 152–160
13. **Lindquist S** (1986) The heat shock response. Annu Rev Biochem **55:** 1151–1191
14. **Lowry OH, Rosebrough NJ, Farr AL, Randall RJ** (1951) Protein measurement with the Folin phenol reagent. J Biol Chem **193:** 265–275
15. **Mansfield MA, Key JL** (1987) Synthesis of the low molecular weight heat shock proteins in plants. Plant Physiol **84:** 1007–1017
16. **McAlister L, Finkelstein DB** (1980) Heat shock proteins and thermal resistance in yeast. Biochem Biophys Res Commun **93:** 819–824
17. **Nagao RT, Czarnecka E, Gurley WB, Schoffl F, Key JL** (1985) Genes for low-molecular-weight heat shock proteins of soybean: sequence analysis of a multigene family. Mol Cell Biol **5:** 3417–3428
18. **O'Farrell PH** (1975) High resolution two-dimensional electrophoresis of proteins. J Biol Chem **250:** 4007–4021
19. **Peterson NS, Mitchell HK** (1981) Recovery of protein synthesis after heat shock: prior heat-treatment affects the ability of cells to translated mRNA. Proc Natl Acad Sci USA **78:** 1708–1711
20. **Schlesinger MJ, Ashburner M, Tissieres A** (1982) Heat Shock: From Bacteria to Man. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
21. **Schoffl F, Key JL** (1982) An analysis of mRNA for a group of heat shock proteins of soybean using cloned cDNA. J Mol Appl Genet **1:** 301–314
22. **Towbin H, Staehelin T, Gordon J** (1979) Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc Natl Acad Sci USA **76:** 4350–4354
23. **Vierling E** (1991) The role of heat shock proteins in plants. Annu Rev Plant Physiol Plant Mol Biol **42:** 579–620.