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Immunological Kinship of Class I Low Molecular Weight Heat Shock Proteins and Thermostabilization of Soluble Proteins in Vitro among Plants [1]

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The antibody prepared against one of the soybean (*Glycine max*) 15 to 18 kDa heat shock proteins (HSPs) that cross-reacted with the 12 polypeptides of 15 to 18 kDa class I low molecular weight (LMW) HSPs in soybean, was also found to cross-react in Western blot analyses with the class I LMW HSPs of nine other plant species, i.e., mung bean, pea, cucumber, tobacco, *Arabidopsis*, rice, maize, wheat, and barley. An antibody raised from the 16.9 kDa rice HSP also cross-reacted with the same class I LMW HSPs of the ten plant species tested. HSPs-enriched fractions (70 to 100% ammonium sulfate saturation) prepared from mung bean and rice heat-shocked seedlings were able to thermostabilize the homologous soluble proteins, as we have shown previously in soybean. Up to 50% of the soluble proteins that are normally denatured by heating at 55°C for 30 min was protected when an HSPs-enriched fraction was added to either mung bean or rice protein. Additionally, the HSPs-enriched fractions were exchangeable among these three plant species for thermostabilization. The protection provided by these HSPs-enriched fractions is effective mainly for membrane-associated proteins. In soybean depletion of the 15 to 18 kDa HSPs in the HSPs-enriched fraction resulted in the loss of the thermostabilizing ability and when the 15 to 18 kDa HSPs were recovered in this fraction, the thermostabilizing ability was again restored. Thus, the 15 to 18 kDa HSPs in plant, which shuttle between the cytoplasm and cellular organelles during heat shock (HS) and recovery from HS, are responsible for providing the thermostabilization.

Key words: Class I low molecular weight heat shock proteins — Heat shock protein — Immunological kinship — Thermostabilization.

Organisms ranging from bacteria to man respond to high temperatures by synthesizing a new set of proteins, the HSPs, and repressing the synthesis of most normal proteins (Schlesinger et al. 1982). The universality and evolutionary conservation of HSPs has led to the view that these proteins play an important fundamental role in organism survival to high temperatures. Among the LMW HSPs ranging in mass from 15 to 28 kDa, the 15 to 18 kDa HSPs are the most abundant proteins induced by heat stress in many plant species (Mansfield and Key 1987). In animals, insects, and yeast cells, in contrast to higher plants, the HMW HSPs of 68 to 110 kDa with 70 kDa HSP are predominant (Lindquist 1986). Although plants do synthesize a similar set of HMW HSPs, most of the translational capacity is devoted to the synthesis of the LMW HSPs (15 to 28 kDa), as measured by incorporation of radioactive amino acids (Mansfield and Key 1987). These proteins accumulate to a significant level after several hours of HS and exhibit a considerable heterogeneity in isoelectric point, molecular weight, and stainability.

While the physiological function of HSPs is unknown, a positive correlation between the synthesis of HSPs and the development of thermotolerance in a time- and temperature-dependent manner suggests that accumulation of HSPs is an essential component of the protection process

Abbreviations: HS, heat shock; HSPs, heat shock proteins; LMW, low molecular weight; HMW, high molecular weight; PAGE, polyacrylamide gel electrophoresis; PMSF, phenylmethyl sulfonylfluoride; PRS, postribosomal supernatant; AS, ammonium sulfate.

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(Gerner and Schneider 1975, Kimple et al. 1990, Li and Werb 1982, Lin et al. 1984, Petersen and Mitchell 1981). Studies on the localization of the HSPs, kinetics of their association with organelles during HS, and dissociation during recovery from HS in soybean seedlings (Lin et al. 1984) further suggest that this dynamic localization process is important for thermoprotection during HS and for resumption of cellular activities after HS. Recently, we demonstrated that isolated soybean mitochondria, when associated with 15 to 18 kDa and some 68 to 70 kDa HSPs, were functional in oxidative phosphorylation in vitro at 42.5°C HS temperature (Chou et al. 1989). We also reported that the HSPs-enriched fraction, obtained by ammonium sulfate fractionation of an extract from heat shocked soybean seedlings, was very stable to heating, and it maintained the solubility of proteins that would otherwise be denatured by heat (Jinn et al. 1989).

In this report, we used two antibodies: one prepared against a soybean 15 to 18 kDa class I LMW HSP (Hsieh et al. 1992) and which cross-reacts with 12 polypeptides in the 15 to 18 kDa class of soybean HSPs and the other prepared against the rice 16.9 kDa HSP (pI 6.4) to test the immunological kinship of class I LMW HSPs among ten different plant species. We also used ammonium sulfate fractionation procedures to prepare an HSPs-enriched fraction (Jinn et al. 1989) to test the exchangeability of HSPs from soybean, mung bean and rice for thermostabilization of soluble proteins. Among these HSPs, we also elucidated which classes of HSPs could provide thermoprotection and what kind of soluble proteins were protected.

Materials and Methods

Plant materials—The following plant species: soybean (*Glycine max* cv. Taita Kaohsiung No. 8), mung bean (*Vigna radiata* L.), rice (*Oryza sativa* L. cv. Tainong No. 67), pea (*Pisum sativum* L.), maize (*Zea mays* L.), wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.), sunflower (*Helianthus annuus* L.), cucumber (*Cucumis sativas* L.) were used in this study. All seeds were surface sterilized in 10% Clorox for 10 min, rinsed thoroughly in water, and then germinated in a roll of moist paper towel at 28°C in a dark growth chamber (Lin et al. 1984). Seedlings of tobacco (*Nicotiana plumbaginifolia* 43C) with 6–8 cm long and *Arabidopsis* (*Arabidopsis thaliana* L.) with 2–3 cm long grown under light were provided by Drs. C.C. Chen and S.M. Pang of this department.

Preparation of postribosomal supernatant—Two-day-old seedlings of soybean and mung bean without cotyledons and five-day-old rice seedlings without endosperm were incubated in incubation buffer (5 mM potassium phosphate buffer, pH 6.0) containing 1% sucrose, 50 μg ml^{-1} chloramphenicol and L-[4,5-^{3}H]leucine or L-[^{35}S]methionine [New England Nuclear, Boston, MA, U.S.A.] at a concentration of 20 μCi ml^{-1} at the indicated temperature with gentle shaking. Seedlings were harvested and homogenized with a polytron in 0.2 M Tris-HCl buffer, pH 8.8, containing 0.5 M sucrose, 0.1 M KCl, 30 mM $MgCl_2$, 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 90 min, as described previously (Jinn et al. 1989) for the preparation of the postribosomal supernatant.

Ammonium sulfate fractionation—The postribosomal supernatants were fractionated by 0 to 70%, and 70 to 100% saturation with ammonium sulfate (AS). The precipitates from each fraction were pelleted, dissolved in 50 mM Tris-HCl buffer, pH 8.8, containing 1 mM EDTA and 0.1% 2-mercaptoethanol, and dialyzed overnight against the same buffer as described previously (Jinn et al. 1989). All experimental procedures were carried out at 4°C.

Cell sap preparation—The cell sap was prepared by thawing soybean seedlings that were previously frozen in liquid nitrogen. Seedlings were centrifuged at 1,000 × *g* for 10 min according to the method of Zudo et al. (1983).

Measurement of ^{3}H- or ^{35}S-labeled proteins and quantitative estimation of proteins—For measurement of ^{3}H- or ^{35}S-labeled proteins, a sample aliquot was blotted on a 3MM filter paper and processed as described by Mans and Novelli (1961). Proteins were measured according to the method of Lowry et al. (1951).

SDS-PAGE and immunoblotting—Total proteins were extracted with SDS extraction buffer at room temperature as described previously (Jinn et al. 1989). One dimensional gel electrophoresis was performed according to Laemmli (1970) using 13.75% (w/v) acrylamide gels. Fluorography of the gels was accomplished using ENHANCER (New England Nuclear, Boston, MA, U.S.A.) and Kodak film (XAR-5). For immunoblotting, proteins were transferred from SDS gels to Immobilon PVDF Transfer Membranes (Millipore) with glycine electrode buffer according to Towbin et al. (1979). Protein bands cross-reacting with the LMW HSPs antibodies were identified by reaction with alkaline phosphatase conjugated to goat anti-rabbit IgG (DAKOPATTS, Denmark). Bound antibodies were visualized by reaction with BCIP and NBT according to the manufacturer's specification (Bio-Rad).

Assay for thermal denaturation of soluble proteins—The HSPs-enriched protein fraction prepared from 70 to 100% ammonium sulfate saturation was added to ^{3}H- or ^{35}S-labeled soluble proteins prepared from the postribosomal supernatant of non-heat shocked seedlings, the mixture was heated at 55°C for 30 min with shaking. The denatured proteins were pelleted at 16,000 × *g* for 15 min and the radioactivity in the pellet was measured after suspension in the Laemmli's sample buffer (Laemmli 1970).

Localization and delocalization of HSPs during HS and recovery from HS—Three samples of soybean seed-

lings were incubated at 40°C for 3 h followed by incubation at 28°C for 4 h. Two of the samples were then shifted to 45°C for 30 min, and one of these two was subsequently transferred to 28°C for 4 h. After each treatment, postribosomal supernatant were prepared from the seedlings. The treatment regime of 40°C (3 h) → 28°C (4 h) → 45°C (30 min) causes an association of HSPs with cellular components, and additional incubation at 28°C for 4 h causes delocalization of HSPs from cellular components and their return to cytosol (Lin et al. 1984).

Antibodies—Preparation of the antibody against a soybean LMW HSP has been described (Hsieh et al. 1992). The antibody against the 16.9 kDa HSP from rice (pI 6.4) was prepared using protein synthesized from a pGEX-2T expression vector carrying the cDNA pTS1 (Tseng 1992).

Results

Immunoreaction—The antibody raised against a single soybean HSP which cross-reacts only with the 15 to 18 kDa class of HSPs in soybean showed a similar immunoreaction pattern to the class I LMW HSPs of dicotyledons (soybean, mung bean, pea, cucumber, tobacco, *Arabidopsis*) and monocotyledons (rice, maize, wheat, and barley) (Fig. 1A). None of the HMW HSPs or 20 to 28 kDa LMW HSPs cross-reacted with the antibody. Antibody prepared against the 16.9 kDa rice HSP gave similar immunoreaction patterns as the antibody against the soybean HSP (Fig. 1B). The antibodies prepared from these two plants' LMW HSPs did not react with HSPs of *Drosophila* (lane 3, Fig. 2A, B). These results show that the class I LMW HSPs of plants share an antigenicity that is not present in other families of HSPs.

Ammonium sulfate fractionation of the postribosomal supernatant—We used the ammonium sulfate fractionation procedure to separate soluble proteins from heat-shocked rice and mung bean seedlings into two fractions: 0 to 70% and 70 to 100% saturation. Fig. 3 A and B show the fluorograms of each fraction from mung bean and rice respectively. Separation by SDS-PAGE revealed that the HSPs, especially the 15 to 18 kDa HSPs, were enriched in the 70 to 100% saturation fraction (lane 6 of Fig. 3A, B), as shown previously in soybean (Jinn et al. 1989). Some of the HMW HSPs in mung bean (lane 4 of Fig. 3A) and rice (lane 2 and 4 of Fig. 3B) were found in other fractions. Among all plant species studied, HSPs were all enriched in the 70 to 100% saturation fraction. The 70 to 100% AS saturation fraction is therefore termed the HSPs-enriched fraction

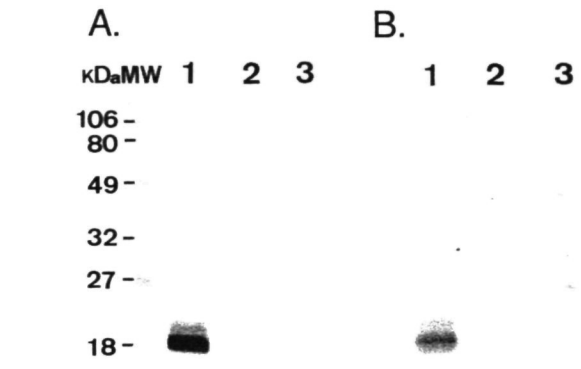

Fig. 2 Western blot analysis of *Drosophila* HSPs using antibodies against soybean HSP (A) and against rice HSP (B). Lane 1, total proteins from heat-shocked soybean seedlings; Lane 2, total proteins from *Drosophila* larvae grown at 25°C. Lane 3, total proteins from *Drosophila* larvae treated at 37°C for 30 min followed by 25°C for 90 min.

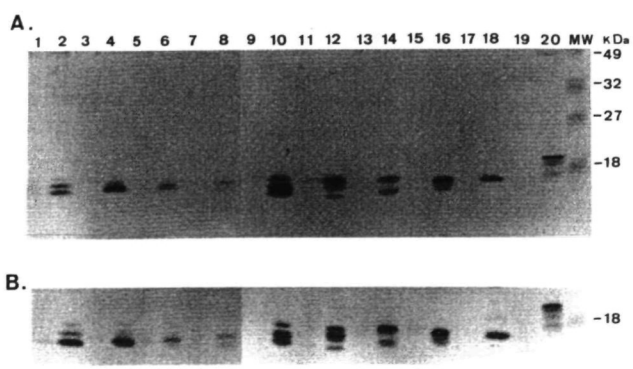

Fig. 1 Western blot analysis using antibody against soybean HSP (A) and antibody against rice HSP (B). 1 and 2, rice; 3 and 4, maize; 5 and 6, wheat; 7 and 8, barley; 9 and 10; soybean 11 and 12, mung bean; 13 and 14, pea; 15 and 16, cucumber; 17 and 18, tobacco; 19 and 20, *Arabidopsis*. The odd numbers are from the control (28°C) and the even numbers are from heat-shocked treatment.

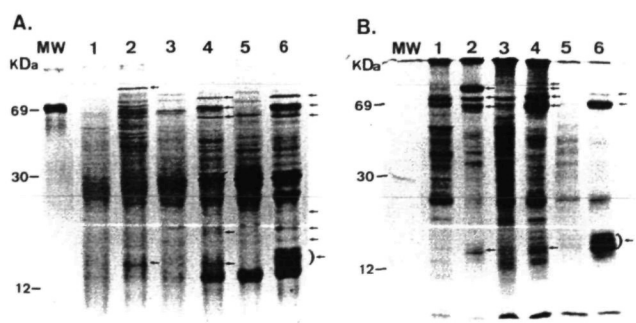

Fig. 3 Fluorograms of SDS-PAGE analysis of proteins from mung bean (A) and rice (B) after AS fractionation. Lane 1 and 2, total proteins before AS fractionation; 3 and 4, 0 to 70% AS saturation; 5 and 6, 70 to 100% AS saturation. 1, 3 and 5 are proteins from 28°C and 2, 4 and 6 are proteins from 40°C. Arrows indicate HSPs. The MW markers are shown in the left lane of Lane 1.

Table 1 Stabilization of ^{3}H-proteins against heat denaturation by addition of HSPs-enriched fractions from soybean, mung bean, and rice

Addition 70–100% AS fraction		Proteins denatured (cpm × 10^{-2})		
		Soybean	Mung bean	Rice
Expt. 1				
soybean	C (28°C)	898 (100%)	2,368 (100%)	—
	HS (40°C)	477 (53.1%)	1,372 (57.9%)	—
mung bean	C	1,610 (100%)	1,414 (100%)	—
	HS	835 (51.8%)	735 (51.9%)	—
Expt. 2				
soybean	C (28°C)	1,750 (100%)	—	2,590 (100%)
	HS (40°C)	1,010 (57.7%)	—	1,000 (38.5%)
rice	C	330 (100%)	—	1,340 (100%)
	HS	230 (69.7%)	—	700 (52.2%)

To 2 mg of ^{3}H-proteins from the postribosomal supernatant, 1 mg of a 70–100% AS fraction (from either 28°C or 40°C treated seedlings) was added; the mixture was heated at 55°C for 30 min. After heat treatment, the samples were centrifuged at 16,000 × *g* for 15 min. The pellets were suspended in 250 μl of Laemmli's sample buffer, and 25 μl duplicate samples were assayed for radioactivity. The amount of ^{3}H-protein that pellets after heating by addition of C (28°C) fraction in this Table and other Tables were found always equal to 98% to 95% of pelletable proteins by heating when nothing was added.

and was used for the thermo-stabilization of soluble proteins.

Stabilization of soluble proteins against heat denaturation by addition of HSPs-enriched fraction—Table 1 shows that HSPs-enriched fractions obtained from each plant species were able to provide protection to soluble proteins of the same species from heat denaturation at 55°C by nearly 50%; that is, some proteins which were normally denatured after heating were protected with addition of the HSPs-enriched fraction. The source of HSPs-enriched fraction is exchangeable for thermostabilizing soluble proteins among the three plant species. Nearly 50% and 30% of the soybean proteins were protected by the HSPs-enriched fractions from mung bean and rice respectively, and more than 60% of the rice soluble proteins and 40% of the mung bean soluble proteins were protected by the HSPs-enriched fraction from soybean. The HSPs-enriched fraction from rice was very effective in protecting its own soluble proteins from heat denaturation; however, this fraction provided a relatively low level of protection to the soluble proteins of soybean (only 30% protected). On the other hand, the HSPs-enriched fraction from soybean provided a higher protection to the rice soluble proteins (up to 61% protected) compared to the protection normally obtained for its own soluble proteins.

Proteins protected by HSPs from heat denaturation—The cell sap proteins as shown in Table 2 are not easily protected from heat denaturation by an HSPs-enriched frac-

Table 2 Thermostabilization of ^{3}H-proteins from cell sap or from the postribosomal supernatant prepared from the residue of the cell sap preparation by addition of the HSPs-enriched fraction from soybean

Addition 70–100% AS fraction	Proteins denatured (cpm)	
	Cell sap	Extract of residues[a]
28°C	7,830±370 (100%)	15,060±780 (100%)
40°C (3 h) → 28°C (3 h)	7,290±330 (93.1%)	8,280±410 (55.0%)

To 1 mg of ^{3}H-cell sap (1.14×10^5 cpm) or the ^{3}H-extract from the residue (7.11×10^4 cpm) proteins, 1 mg of the 70–100% AS fraction from seedlings treated under one of two different temperature regimes was added; the mixtures were then heated at 55°C for 30 min. After the heat treatment, the mixtures were centrifuged at 16,000 × *g* for 15 min. The pellets were suspended in 250 μl of Laemmli's sample buffer, and 25 μl duplicate samples were assayed for radioactivity. Each result is presented as the mean ± standard error of the mean.

[a] Proteins were extracted from the residue of the cell sap preparation.

Table 3 Thermostabilization of ^{3}H-proteins in a low or high salt extract by addition of HSPs-enriched fraction from soybean

Addition 70–100% AS fraction	Proteins denatured (cpm)	
	Low salt extract [a]	High salt extract from residue of low salt extract [b]
No addition	17,130±670 (100%)	12,130±410 (100%)
28°C	16,230±600 (94.7%)	12,180±380 (100%)
40°C (3 h) → 28°C (3 h)	15,890±490 (92.7%)	9,110±210 (75.1%)

To 750 μg of the postribosomal supernatant proteins from a low salt extract (62,000 cpm) or from a high salt extract of the residues from the low salt extract (55,890 cpm), 750 μg of the 70–100% AS fraction from seedlings of two different treatments were added; the mixtures were then heated at 55°C for 30 min. The salt concentration in the assay mixtures from the low salt extract was adjusted to the same concentration as in the high salt extract. After heat treatment, the samples were centrifuged at 16,000 × *g* for 15 min. The pellets were suspended in 250 μl of Laemmli's sample buffer, and 25 μl duplicate samples were assayed for radioactivity. Each result is presented as the mean ± standard error of the mean.

[a] The low salt extraction medium contained 50 mM Tris-HCl buffer, pH 8.8, 0.5 M sucrose, 50 mM KCl, 5 mM $MgCl_2$, 1 mM DTT, and 1 mM PMSF.

[b] The residues were extracted with the medium as described in Methods.

tion. In contrast, the HSPs-enriched fraction can thermostabilize soluble proteins from an extract of the residues from the cell sap preparation (45% protected). High-salt extractable proteins from the postribosomal supernatant, which are typically considered to be membrane-associated, can be somewhat protected by a HSPs-enriched fraction (up to 25%), while the low-salt extractable proteins are not (Table 3). The SDS profile of proteins that are thermoprotected in the postribosomal supernatant is shown in Fig. 4; lanes 4 and 5 are the undenatured proteins that remained in the supernatant after heating; lanes 2 and 3 are the denatured proteins in the pellets, and lane 1 is the profile of proteins in the postribosomal supernatant.

Fluorograms of 70 to 100% AS saturation fraction and western blot of four different temperature treatments—Fig. 5A (fluorogram) and Fig. 5B (western blot) show the 70 to 100% AS saturation fractions from soybean seedlings treated with four different temperature regimes. Lane 3 is a treatment which we have shown previously results in the association of the 15 to 18 kDa HSPs and a small fraction of the 68 to 70 kDa HSPs with the organellar fraction, so the postribosomal supernatant from which the AS fraction is prepared, is thus deficient in the 15 to 18 kDa HSPs but still contains the HMW HSPs. The treatments profiled in lanes 2 and 4 demonstrate that the 15 to 18 kDa HSPs are delocalized from organellar components and returned to cytosol after a 4 h recovery at 28°C. The western blot analysis shown in Fig. 5B confirmed that the 15 to 18 kDa LMW HSPs of Fig. 5A are HSPs.

Protecting ability of HSPs other than the 15- to 18-kDa HSPs—We then tested the ability of an HSPs-enriched fraction that was depleted of the 15 to 18 kDa HSPs to thermostabilize soluble proteins. As shown in Table 4, an HSPs-enriched fraction from the 40°C (3 h) → 28°C (4 h) treatment protected approximately 50% of postribosomal supernatant proteins; while a fraction from the 40°C (3 h) → 45°C (30 min) treatment, which is depleted of the 15 to 18 kDa HSPs (Fig. 5 lane 3) did not provide any protection,

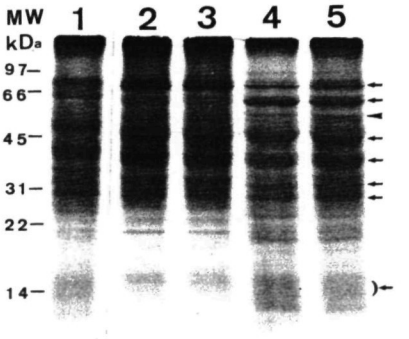

Fig. 4 Fluorogram of the normal ^{35}S-labeled proteins protected against heat denaturation. Lane 1, total proteins of the postribosomal supernatant; Lane 2, denatured proteins (in pellets) from the postribosomal supernatant; after heating at 55°C for 30 min, with addition of 70 to 100% AS saturation fraction from 28°C control seedlings; Lane 3, denatured proteins (in pellets) from the postribosomal supernatant; after heating at 55°C for 30 min, with addition of 70 to 100% AS saturation fraction from 40°C (3 h) → 28°C (3 h) treated seedlings; Lane 4 and 5 represent proteins which remained in the supernatant after heating of the mixtures in Lane 2 and 3 respectively. Lanes 2, 3, 4, and 5 were loaded with equal cpm from Laemmli's sample buffer. Arrows and arrow head indicate quantitative and qualitative differences, respectively.

Table 4 Thermostabilization of ^{3}H-proteins by addition of a HSPs-enriched fraction prepared from one of four different temperature treatments of soybean seedlings

Addition 70–100% AS fraction	Proteins denatured (cpm)
No addition	186,880±1,020 (100%)
28°C (3 h)	182,360± 720 (97.6%)
40°C (3 h) → 28°C (3 h)[a]	105,100±1,290 (56.3%)
40°C (3 h) → 45°C (30 min)[b]	180,650± 980 (96.7%)
40°C (3 h) → 45°C (30 min) → 28°C (4 h)[c]	97,650±1,120 (52.3%)

To 2 mg of ^{3}H-proteins from the postribosomal supernatant (675,400 cpm), 1 mg of the 70 to 100% AS fraction from seedlings of four different treatments was added; the mixtures were heated at 55°C for 30 min. After the heat treatment, the samples were centrifuged at 16,000 × *g* for 15 min. The pellets were suspended in 250 μl of Laemmli's sample buffer, and 25 μl duplicate samples were assayed for radioactivity. Each result is presented as the mean ± standard error of the mean.

[a] The subsequent treatment at 28°C for 3 h cause the partial HSPs associated with organelles to redistribution into the cytosol (Lin et al. 1984).

[b] Under this treatment regime, many LMW HSPs remain associated with organelles (Lin et al. 1984).

[c] Under this treatment regime, HSPs are redistributed into the cytosol (Lin et al. 1984).

even with the presence of HMW HSPs. Although these treatments were done previously and we confirmed the earlier findings (Jinn et al. 1989), in this study we did additional treatment shown in Table 3 lane 5 for the relocalization of the associated 15 to 18 kDa HSPs with organelles in AS fraction. In the 40°C (3 h) → 45°C (30 min) → 28°C (4 h) treatment, the 15 to 18 kDa HSPs were recovered in 70 to 100% AS saturation fraction (Fig. 5 lane 4); concomitantly, the thermostabilization ability was restored and some 50% of postribosomal supernatant proteins were again protected. These data suggest that only the 15 to 18 kDa HSPs, present in 70 to 100% AS saturation fractions are required for thermoprotection of soluble proteins.

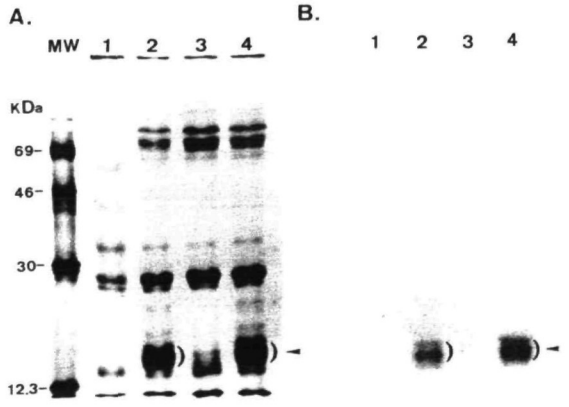

Fig. 5 Fluorogram (A) and Western blot (B) of 70 to 100% AS fractions from four different treatments of soybean seedlings analyzed separate by SDS-PAGE. Lane 1, 28°C (3 h); Lane 2, 40°C (3 h) → 28°C (3 h); Lane 3, 40°C (3 h) → 45°C (30 min); Lane 4, 40°C (3 h) → 45°C (30 min) → 28°C (3 h). In lane 2, 3 and 4 were loaded with equal cpm (Fig. A) or equal amount (Fig. B) of protein. Arrow head indicates the 15 to 18 kDa HSPs.

Discussion

All plants synthesize LMW HSPs ranging in size from 15 to 28 kDa, and in many species the 15 to 18 kDa class is among the most abundant proteins induced by heat stress (Mainsfield and Key 1987). LMW HSPs genes have been cloned from soybean (Nagao et al. 1985, Raschke et al. 1988, Schöffl and Key 1982, 1983), pea (Lauzon et al. 1990), lily (Bouchard 1990), *Arabidopsis* (Helm and Vierling 1989, Takahashi and Komeda 1989), carrot (Zimmerman et al. 1989), petunia (Chen and Vierling 1991), rice (Tseng et al. 1992), maize (Goping et al. 1991), wheat (McElwain and Spiker 1989), and *Chlamydomonas* (Grimm et al. 1989). Analyses of these genes indicate that most of the LMW HSPs belong to four multi-gene families (Vierling 1991). Two of these gene families most likely encode cytoplasmic proteins, with one encoding a chloroplast-localized protein and the other an endomembrane protein. The percentage of the similarity of amino acid sequence is shown to be high for the class I cytoplasmic LMW HSPs genes of soybean, pea, and *Arabidopsis*, ranging from 80.1% to 92.9% with the highest value between soybean and pea (Helm and Vierling 1990). Thus, it was not surprising to find that antibodies raised against a class I LMW HSPs from either soybean or rice cross-reacted with the LMW HSPs from mung bean, pea, cucumber, tobacco, *Arabidopsis*, maize, wheat, and barley. The fact that this class of proteins is unique and is underscored by the lack of cross-reactivity of either of these class I HSP antibodies with other classes LMW HSPs from plants or with HSPs

from *Drosophila*.

In addition to this conserved antigenicity, we demonstrate here that these proteins have a conserved AS fractionality. In the presence of these proteins, soluble proteins which would otherwise become insoluble upon heat treatment remain soluble. We reported recently that an HSPs-enriched protein fraction prepared from heat-shocked soybean seedlings provided thermostabilization of normal soluble proteins. The degree of protection was proportional to the amount of the HSPs-enriched fraction added (Jinn et al. 1989). These experiments were carried out based on the model proposed for the biological role of HSPs by Minton et al.(1982), which suggests that HSPs may act as heat stable proteins to nonspecifically stabilize other proteins which are highly susceptible to inactivation or denaturation by heat. Under our standard assay condition, some 50% of the soluble proteins that are normally denatured by heating at 55°C for 30 min was protected when the HSPs-enriched fraction was added. When the temperature was subsequently decreased to more natural physiological conditions by heating at 35°C up to 16 h or 40°C for 9 h, the HSPs-enriched fraction provided the same effect, protecting about 50% of the soluble protein from heat denaturation (Wu 1992). In soybean, mung bean, and rice, almost all the HSPs are enriched in 70 to 100% AS saturation fraction, which is not surprising since in the hydropathy profiles derived from amino acid sequences show that the class I HSPs are quite hydrophilic (Czarnecka et al. 1985).

The results present here further characterized the thermostabilizing ability of the 70 to 100% AS fraction from heat shocked seedlings of soybean, mung bean and rice. Generally, the HSPs-enriched fraction prepared from either mung bean or rice could provide protection to its own soluble proteins as effectively as that from soybean (Jinn et al. 1989). The HSPs-enriched fraction prepared from heat-shocked rice seedlings provided protection of soybean soluble proteins from heat denaturation with relatively low efficiency compared to the soybean HSPs-enriched fraction's ability to protect rice soluble proteins (Table 1). These different protection abilities between dicots and monocots probably reflect the difference in the actual amounts of HSPs in each preparation of HSPs-enriched fraction, rather than a difference in functional activity of HSPs themselves. According to our previous experiments, the 15 to 18 kDa HSPs in soybean accumulate to a detectable amount by Coomassie blue stain (Jinn et al. 1989, Hsieh et al. 1992), but accumulate much less in rice (data not shown).

The results presented in Fig. 5 and Table 4, strongly suggest that the 15 to 18 kDa HSPs are responsible for the thermostabilization. This conclusion is further supported by a study using the 16.9 kDa rice HSP synthesized with a recombinant vector. This protein provided very impressive thermostabilization for soluble proteins, and the degree of protection was proportional to the amount of this protein added; up to 70% of soluble proteins could be protected (Wu 1992). In our standard assay, a final concentration of KCl up to 300 mM did not affect the thermostabilization by HSPs (Wu 1992). Therefore, the differences in the solubility response upon addition of AS fraction was due to differences in proteins extracted by the different salt concentrations, rather than an effect of salt concentrations on heat denaturation. The proteins protected by the HSPs-enriched fractions are not the cytosolic proteins but are the higher ionic strength extractable proteins, and they are probably membrane-associated proteins. This observation correlates well with the localization of HSPs with organelles during HS (Lin et al. 1984).

Although the physiological function of HSPs remains unknown, we have shown previously that synthesis and accumulation of LMW HSPs and their cellular localization are strongly correlated with the acquisition of thermotolerance in soybean seedlings (Lin et al. 1984). We have also demonstrated that isolated mitochondria, when associated with the 15 to 18 kDa class of HSPs, can proceed with oxidative phosphorylation in vitro at high HS temperature (Chou et al. 1989). In studies presented here, along with our earlier study on the soybean 15 to 18 kDa HSPs-enriched fraction, further establish a potentially important role for this class of HSPs in the protection of plants from thermal stress. It will be interesting to discover the in vivo significance of these observation on the ability of class I LMW HSPs to stabilize protein solubility in vitro.

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