Heat Shock Inhibits α-Amylase Synthesis in Barley Aleurone without Inhibiting the Activity of Endoplasmic Reticulum Marker Enzymes

Liliane Sticher1, Asok K. Biswas2, Douglas S. Bush, and Russell L. Jones*
Department of Plant Biology, University of California, Berkeley, California 94720

ABSTRACT

The effects of heat shock on the synthesis of α-amylase and on the membranes of the endoplasmic reticulum (ER) of barley (Hordeum vulgare) aleurone were studied. Heat shock, imposed by raising the temperature of incubation from 25°C to 40°C for 3 hours, inhibits the accumulation of α-amylase and other proteins in the incubation medium of barley aleurone layers treated with gibberellic acid and Ca2+. When ER is isolated from heat-shocked aleurone layers, less newly synthesized α-amylase is found associated with this membrane system. ER membranes, as indicated by the activities of NADH cytochrome c reductase and ATP-dependent Ca2+ transport, are not destroyed by heat stress, however. Although heat shock did not reduce the activity of ER membrane marker enzymes, it altered the buoyant density of these membranes. Whereas ER from control tissue showed a peak of marker enzyme activity at 27% to 28% sucrose (1.119–1.120 grams per cubic centimeter), ER from heat-shocked tissue peaked at 30% to 32% sucrose (1.127–1.137 grams per cubic centimeter). The synthesis of a group of proteins designated as heat-shock proteins (HSPs) was stimulated by heat shock. These HSPs were localized to different compartments of the aleurone cell. Several proteins ranging from 15 to 30 kilodaltons were found in the ER and the mitochondrial/plasma membrane fractions of heat-shocked cells, but none of the HSPs accumulated in the incubation medium of heat-shocked aleurone layers.

The response of living cells to elevated temperature appears to be widespread and is reflected in the synthesis of a group of highly conserved proteins referred to as HSPs1 (18). The response of the barley aleurone to heat shock is typical of the response of other plant tissues to elevated temperature (3, 4, 22). When aleurone layers treated with GA3 and Ca2+ and incubated at 25°C are transferred to 40°C, the pattern of protein synthesis is altered. Whereas the synthesis of α-amylase and other secreted enzymes is inhibited by heat shock, the synthesis of HSPs is induced by elevated temperature (3). In barley aleurone, heat shock inhibits the synthesis of proteins such as α-amylase by causing rapid degradation of the α-amylase mRNA (3). Resumption of the synthesis of α-amylase and other proteins upon transfer of aleurone tissue from 40°C to 25°C also requires the synthesis of RNA, lending further support to the hypothesis that RNA degradation occurs during heat-shock treatment (3).

Aleurone layers exposed to high temperature also show altered endomembrane organization. In particular, Belanger et al. (3), using EM, noted that rough ER, which is particularly abundant in aleurone cells incubated with GA3 and Ca2+ at 25°C (10), is reduced by about 75% in heat-shocked aleurone layers. They proposed that ER plays an important role in stabilizing α-amylase mRNA in aleurone cells. They argued that, since α-amylase is a secreted protein that is synthesized on membrane-bound ribosomes, the stability of mRNA for this protein is a consequence of its binding to the endomembrane system (3, 4).

Although Belanger et al. (3) found a strong correlation between the impaired synthesis of secreted proteins and the abundance of ER in heat-shocked barley aleurone, there is no consensus that heat shock regulates the synthesis of all ER-associated proteins or causes the destruction of mRNA or ER in all plant cells. In Phaseolus vulgaris cotyledons, heat shock enhances the synthesis of PHA, a protein-body protein, and impeded its transport out of the ER but does not destroy the ER (7). A similar observation has been made in soybean cotyledons where heat stress enhanced the synthesis of the major storage proteins conglycinin and glycinin (1).

Because of our interest in intracellular transport of α-amylase, we investigated the effect of heat shock on the endomembrane system and on the synthesis of secretory proteins in the barley aleurone layer. In this paper, we report on the effect of elevated temperature on the incorporation of radiolabeled amino acids into membrane-associated proteins. We show that heat shock inhibits the synthesis of secreted proteins and enhances the synthesis of HSPs localized within several intracellular compartments. We found that the levels of ER-specific marker enzyme activities are not reduced by heat shock and conclude that barley aleurone ER is not destroyed by heat-shock treatment.

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2 Present address: Friedrich Miescher-Institut, Basel, Switzerland.
3 Present address: Department of Botany, University of Calcutta, Calcutta, India.
4 Abbreviations: HSP, heat-shock protein; BTP, bis-tris [(bis-[2-hydroxyethyl]iminio)-tris-hydroxymethyl]methane; MT/PM, mitochondrial/plasma membrane; PHA, phytohemagglutinin; Met, methionine; IDPase, inosine diphosphatase; CCR, Cyt c reductase; IEF, isoelectric focusing; pl, isoelectric point.
MATERIALS AND METHODS

Plant Material

Barley grains (Hordeum vulgare L., cv Himalaya, 1985 harvest, Department of Agronomy, Washington State University, Pullman, WA) were de-embryonated and allowed to imbibe water as described elsewhere (14). Aleurone layers were isolated according to Chrispeels and Varner (8) and incubated in 5 μM GA3 (Sigma Chemical Co., St. Louis, MO) and 10 mM CaCl2 (20 aleurone layers per ml medium) for 16 h at 25°C.

Heat-Shock Treatment and Radiolabeling

Aleurone layers were heat-shocked as described by Belanger et al. (3). After incubation in GA3 and CaCl2 at 25°C for 16 h, layers were transferred to fresh incubation medium. For heat-shock treatment layers were incubated in a water bath at 40°C for 3 h; control layers were maintained at 25°C for 3 h, except for one experiment in which aleurone layers were pulse-labeled with [35S]Met (specific activity 3.7 × 1013 Bq/mm, Amersham, Arlington Heights, Ill.; 7.4 × 104 Bq per aleurone layer) for the duration of the heat-shock or control incubation. For that experiment, aleurone layers were incubated at 25°C or 40°C for 30 min before addition of [35S]Met. For all radiolabeling experiments 7.4 × 104 Bq [35S]Met per aleurone layer was used except for one experiment in which 1.5 × 104 Bq [35S]Met per layer was used.

Homogenization and Cell Fractionation

Aleurone layers were homogenized and subcellular fractions isolated and analyzed as summarized in Figure 1. Aleurone layers (100) were homogenized with a motorized razor blade chopper (13) in 7 mL of 25 mM Heps buffered to pH 7.4 with BTP containing 3 mM EDTA, 1 mM DTT, and either 0.5% BSA or 1 mM KBrO3. The homogenate was filtered through two layers of Miracloth (Calbiochem, La Jolla, CA) and centrifuged at 1000g in a Sorvall SS34 rotor (Du Pont Instruments, Newton, CT) at 2°C for 10 min.

The 1000g supernatant was centrifuged on a discontinuous sucrose gradient composed of 1 mL 50% (w/w) sucrose overlaid with 5 to 10 mL 13% (w/w) sucrose for 2 h at 2°C at 70,000g in SW 27.1 buckets and SW 27 rotor (Beckman). After centrifugation, proteins remaining in the load fraction of the gradient were referred to as cytoplasmic, while the turbid band at the 13% and 50% sucrose interface was referred to as the microsomal fraction (Fig. 1A). The microsomal fraction was collected and layered over a continuous 18% to 45% (w/w) sucrose gradient, and centrifuged for 14 h at 70,000g in the same tubes and rotor. Eighteen 1-mL fractions were collected with an Auto Densi-flow gradient fractionator (Buchler Instruments, Fort Lee, NJ). These fractions were referred to as purified microsomal membranes. All sucrose gradient fractions contained 0.1 mM DTT and were buffered with 25 mM Heps-BTP (pH 7.4). Sucrose concentrations were measured refractometrically.

An exception to the above purification procedure is an experiment in which the 1000g supernatant was loaded di-

![Figure 1. Flow diagram for the isolation of subcellular fractions (A) and tissue extract (B) of barley aleurone layers.](www.plantphysiol.org)

TCA Precipitation

Radioactivity in purified microsomal membranes, microsomal and cytoplasmic fractions, incubation media, and extracts was determined after TCA precipitation using a filter disc method (19). Radioactivity was counted by immersing filter paper strips in a liquid scintillation cocktail consisting of 25% (v/v) Triton X-114 (Sigma), 75% xylenes (Malinckrodt, Paris, NY), and 0.3% (v/v) PPO (Sigma) and counted in an LS-7000 scintillation counter (Beckman).

Enzyme and Protein Assays

α-Amylase (EC 3.2.1.1) was assayed using the standard I$_2$KI procedure (15) and detergent-solubilized, and latent IDPase

Figure 1. Flow diagram for the isolation of subcellular fractions (A) and tissue extract (B) of barley aleurone layers.
was assayed according to the method of Shore and MacLachlan (24) with the following modification for latent IDPase: fractions were assayed after 4 d storage at 4°C, and digitonin was omitted from the assay medium. CCR (EC 1.6.2.1) was assayed as described elsewhere (11). Protein was determined using the Bradford reagent (Bio-Rad) with BSA as a standard.

Ca²⁺ Transport

Net Ca²⁺ uptake into microsomal membranes was monitored using the methods described by Bush and Sze (6) with the modifications of Bush et al. (5). A sample of the purified microsomal membrane fraction (50 μL, approximately 100 μg/ml protein) was mixed with 400 μL of a medium containing (final concentrations) 25 mM Hepes (pH 7.4), 10 mM potassium oxalate, 3 mM MgSO₄, 10 μM CaCl₂, and 100 μM sodium azide and 4μCaCl₂ (3.7 × 10⁸ Bq/μL; specific activity 5.6 × 10¹⁰ Bq/mmols, Amersham) in the presence and absence of 1 mM ATP. After 20 min incubation, the reaction mixture was removed and filtered through 0.45-μm filter discs (type HA, Millipore) under vacuum. The filters were washed with 3.5 mL buffer containing 250 mM sucrose, 2.5 mM Hepes-BTP (pH 7.0), and 0.2 mM CaCl₂, dried, and immersed in scintillation fluid as described by Bush et al. (5). Ca²⁺ transport was calculated as the mean difference between ⁴⁴Ca²⁺ accumulation in the presence and absence of 1 mM ATP.

Gel Electrophoresis

For SDS-PAGE, density gradient fractions were precipitated with 80% (v/v) aqueous ethanol, the precipitate was collected by centrifugation in an Eppendorf Microfuge (Brinkman Instruments, Westbury, NY), and the pellet was resuspended in SDS-PAGE sample buffer. SDS-PAGE was performed in a mini-gel system (6 × 9 × 0.08 cm) using 12% acrylamide and the conditions described by Laemmli (16). Molecular weights were estimated from the position of pre-stained marker proteins (Bio-Rad).

IEF

IEF was performed on Servalyt Precotes pH 3 to 10 (Serva, Heidelberg, FRG) according to the manufacturer’s instructions.

Fluorography

For fluorography, SDS-PAGE and IEF gels were fixed in methanol:acetic acid:water (45:10:45, v/v/v), washed briefly in methanol:acetic acid (50:50, v/v), soaked in fluor (950 mL methanol, 50 g methylamphithalene in 50 mL glacial acetic acid, 5 g 2,5-diphenyloxazole) for 40 min, immersed in water for 20 min to precipitate the fluor, and dried prior to exposure to X-omat AR x-ray film (Eastman Kodak, Rochester, NY).

RESULTS

Protein Synthesis

Heat shock inhibited incorporation of radiolabel into TCA-insoluble proteins in incubation media and in the cytoplasmic and microsomal fractions of aleurone homogenates separated on a discontinuous sucrose density gradient (Table 1). The extent to which heat shock inhibited protein synthesis in these fractions varied with the duration of incubation at 25°C or 40°C (Table 1). Whereas heat shock inhibited label incorporation into the microsomal fraction by 62% after 20 min of incubation, it inhibited protein secretion, as indicated by the accumulation of radioactivity in the incubation medium, by only 15% (Table 1). By 120 min of incubation at 40°C, however, protein synthesis in the microsomal fraction was inhibited by 78% and secretion of labeled proteins by 55% (Table 1). Heat shock also inhibited the synthesis of cytoplasmic proteins by 51% after 2 h at 40°C. The inhibition of protein synthesis induced by heat shock was not due to decreased uptake of [³⁵S]Met, rather the elevated temperature stimulated uptake of [³⁵S]Met by as much as 104% and 38% relative to controls after 20 min and 120 min of incubation respectively (Table 1).

Synthesis and Secretion of α-Amylase

Heat shock had a marked effect on the synthesis of α-amylase and on the accumulation of this protein in the incubation medium. After a 3 h treatment at 40°C, microsomal membranes of aleurone cells had 58% less α-amylase activity (24.4 units) than controls (41.8 units). The incubation medium of layers incubated at 40°C also contained less α-amylase activity than controls. Whereas the medium of aleurone layers incubated for 3 h at 25°C contained 81 units of α-

Table 1. Incorporation of [³⁵S]Met into Microsomal, Cytoplasmic, and Secreted Fractions of Control and Heat-Shocked Aleurone Layers

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Control (25°C)</th>
<th>Heat Shock (40°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>20 min</td>
<td>120 min</td>
</tr>
<tr>
<td>Microsomal</td>
<td>113</td>
<td>298</td>
</tr>
<tr>
<td>Cytoplasmic</td>
<td>98</td>
<td>191</td>
</tr>
<tr>
<td>Incubation medium</td>
<td>473</td>
<td>741</td>
</tr>
<tr>
<td>Crude homogenate</td>
<td>5300</td>
<td>7815</td>
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</tbody>
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amylase, the medium from the same number of layers incubated at 40°C contained only 25 units of amylase activity.

A pulse-chase labeling experiment was conducted to establish whether heat shock influenced directly the secretion of proteins from aleurone cells. Aleurone layers were incubated in \(^{35}\)S-labeled Met for 3 h at 25°C then transferred to a medium hot TCA-precipitable proteins in tissue were low levels of radiolabeled proteins in the incubation medium after 30 min at 40°C, radiolabeled proteins did not accumulate within the tissue. Rather, the level of radiolabeled proteins in extracts declined with time of chase (Fig. 2).

**Heat Shock and the Endomembrane System**

Microsomal membranes isolated from control and heat-shocked aleurone tissue were purified by isopycnic density gradient centrifugation, and the Golgi apparatus and ER membranes were identified by the activities of IDPase (Golgi, Fig. 3) and CCR (ER, Fig. 3) and ATP-dependent Ca\(^{2+}\) transport (ER, Table II). Heat-shock treatment caused a marked reduction in the activity of IDPase (Fig. 3). There were low levels of latent and detergent-soluble IDPase in purified microsomal membranes isolated from heat-shocked aleurone tissue. Membranes from control tissue, on the other hand, had two prominent peaks of IDPase, one at 18% sucrose the other at 28 to 29% sucrose (Fig. 3).

The activity of CCR was not inhibited by heat-shock treatment, rather in all experiments CCR activity was higher in purified microsomal membranes isolated from heat-shocked cells (Fig. 3). The peak of CCR activity in membranes isolated from heat-shocked tissue was always at a higher density (30–32% sucrose) than the peak of CCR activity in membranes from control tissue (27–28% sucrose) (Fig. 3). Note, however, that the buoyant density of ER isolated from aleurone cells in the presence of BSA (Fig. 4) is always higher than the density of ER isolated in the absence of BSA (Fig. 3). We now routinely add BSA to the membrane isolation buffer because ER isolated in the presence of BSA transports \(^{40}\)Ca\(^{2+}\) much more efficiently than ER isolated in its absence (DS Bush, RL Jones, unpublished results). The shift in ER density following heat shock was observed whether membranes isolated in the presence or absence of BSA (Figs. 3 and 4). The level of ATP-dependent Ca\(^{2+}\) transport in the ER fraction of purified microsomal membranes was similar in heat-shocked and control tissues. In the same experiment CCR levels were 121% of controls (Table II).

When purified microsomal membranes were isolated from aleurone layers pulse-labeled with \(^{35}\)S-Met for 3 h at 25°C, radioactivity was incorporated into TCA-precipitable proteins in a broad region of the density gradient with two peaks corresponding to the positions of the ER and MT/PM (Fig. 4) (11). In membranes isolated from heat-shocked tissue, incorporation of \(^{35}\)S-Met into TCA-precipitable proteins in the ER region of the gradient was reduced considerably, but incorporation of \(^{35}\)S-Met into proteins in the MT/PM region was much less affected by heat treatment (Fig. 4).

The distribution of \(\alpha\)-amylase activity in purified microsomal membranes isolated from control tissue was similar to the distribution of CCR (Fig. 4A, C). These enzyme activities peaked in the region of 28–33% sucrose in aleurone layers incubated at 25°C. There was no detectable peak of \(\alpha\)-amylase in purified microsomal membranes from heat-shocked aleurone tissue, on the other hand (Fig. 4C).

SDS-PAGE of density gradient fractions followed by fluo-
Figure 3. Activities of the Golgi apparatus marker enzyme iDPase and the ER marker enzyme CCR in purified microsomal membranes isolated from control (25°C) and heat-shocked (40°C) aleurone layers. iDPase was measured in membranes allowed to stand for 4 d at 4°C (latent iDPase) and after detergent solubilization.

Table II. Effect of Heat Shock on the Activity of α-Amylase and ER Marker Enzymes in the ER Fraction of Purified Microsomal Membranes Isolated from Barley Aleurone Layers

<table>
<thead>
<tr>
<th>Treatment</th>
<th>α-Amylase Activity</th>
<th>ATP-dependent Ca2⁺ Transport</th>
<th>NADH CCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (25°C)</td>
<td>5.6 ± 0.2</td>
<td>59.7 ± 2.7</td>
<td>4.2 ± 0.16</td>
</tr>
<tr>
<td>Heat shock (40°C)</td>
<td>0.6 ± 0.2</td>
<td>57.4 ± 1.9</td>
<td>5.1 ± 0.42</td>
</tr>
</tbody>
</table>

...ography confirmed the observation that heat shock inhibited the synthesis of α-amylase and other proteins (Fig. 5). ER fractions from heat-shocked aleurone layers showed reduced levels of radiolabel in a protein having the same Mr as purified barley α-amylase (Fig. 5). Almost all newly synthesized proteins that were localized in the ER and secreted into the medium were also less heavily labeled in heat-shocked cells (Fig. 5).

The synthesis of several membrane-associated proteins was stimulated by heat shock. Newly synthesized proteins between Mr 10,000 and 30,000 were found in both the ER and MT/PM fractions of heat-shocked cells (Fig. 5). The most prominent of these labeled proteins had an Mr of 30,000 and was located primarily in the MT/PM membrane fractions. Several other labeled proteins with lower Mr were found in either ER or MT/PM fractions and a group of proteins with Mr around 19,000 were found in all microsomal fractions following heat shock treatment of aleurone layers (Fig. 5).

IEF of sucrose density gradient fractions showed that heat shock inhibited the synthesis of most α-amylase isoforms associated with the endomembrane system of the aleurone cell (Fig. 6). With the exception of a protein with a pI of 5.2, the labeling of α-amylase and other proteins in the endomembrane system is considerably reduced by heat shock (Fig. 6).

DISCUSSION

Our data support the observations made by Belanger et al. (3) on the effects of heat shock on protein synthesis in barley aleurone. We have confirmed that heat shock inhibits the synthesis of α-amylase and other secreted proteins and stimulates the synthesis of HSPs in aleurone layers. We have been unable to show that elevated temperature causes the destruction of ER as reported by Belanger et al. (3), however. Rather, using organelle isolation techniques we have shown heat-shocked aleurone cells contain more ER-specific enzyme activities than control layers incubated at 25°C.

Our experiments establish that heat shock inhibits the synthesis of all isoforms of α-amylase and the secretion of these proteins into the incubation medium (Figs. 5 and 6). Several types of experiments indicate that secretion of α-amylase and other proteins from heat-shocked aleurone cells is regulated at the level of the synthesis of these proteins. Pulse-labeling experiments show that heat shock affects pro-
EFFECT OF HEAT SHOCK ON \( \alpha \)-AMYLASE AND ER

**Figure 4.** NADH CCR activity (A), TCA-precipitable radioactivity (B), and \( \alpha \)-amylase activity (C) in purified microsomal membranes isolated from control (25°C) and heat-shocked (40°C) aleurone layers. The regions of the sucrose density gradient enriched in ER and MT/PM are indicated.

Protein synthesis before it affects protein secretion (Table I). In addition, pulse-chase experiments show that heat shock does not cause the accumulation of labeled proteins within the cell (Fig. 3). Organelle isolation experiments confirm that \( \alpha \)-amylase and other secreted proteins do not accumulate in the endomembrane system of heat-shocked aleurone cells.

Rather, the levels of radiolabeled proteins and of \( \alpha \)-amylase activity are much lower in ER fractions isolated from heat-shocked cells (Figs. 4 and 5).

Our results on the effects of heat shock on the membranes of the ER of aleurone cells also contrast with the conclusions of Belanger et al. (3). They reported that heat shock caused destruction of ER, and they advanced the hypothesis that ER destruction was responsible for the reduction in levels of \( \alpha \)-amylase mRNA. We found no evidence for the disappearance of ER following heat stress. When ER was estimated from the
activity of the marker enzyme CCR (Figs. 3 and 4; Table II), the amounts of this membrane were always higher in heat-shocked tissue. When ER was estimated from the activity of an ER-specific ATP-dependent Ca\(^{2+}\) transport ATPase (5), it was not changed following incubation of aleurone tissue at 40°C for 3 h (Table II).

Differences in the methods used to estimate ER membrane levels may explain the different interpretations of the effects of heat shock on the ER of aleurone cells. Whereas our experiments relied on the activities of marker enzymes to measure ER, Belanger et al. (3) used EM. Previous work from our laboratory has shown that EM may not provide an accurate means of quantitating ER membranes in the aleurone cell (12). We propose that the changes in ER observed by Belanger et al. (3) may reflect altered ER organization and not reduced levels of this membrane system. A similar change in ER organization in barley aleurone that is not accompanied by a quantitative change in ER marker enzyme levels (12) has been reported in barley aleurone following treatment with GA\(_3\) (10, 20, 21, 26).

An earlier study from our laboratory on the effects of water stress on the synthesis of \(\alpha\)-amylase in the aleurone layer also indicated that the ER was an important component of stress-induced inhibition of protein synthesis (2). In aleurone layers water-stressed by the addition of polyethylene glycol, inhibition of \(\alpha\)-amylase synthesis was accompanied by the loss of ribosomes from the surface of the ER without a change in the amount of ER membrane (2).

Heat shock increased the buoyant density of the ER membrane system by about 2% sucrose or 0.01 g cm\(^{-3}\) (Figs. 3 and 4). A similar although larger effect of heat shock on the density of ER has been noted in cotyledonary cells of *P. vulgaris* where the density of membranes that synthesize PHA increase from 1.14 g cm\(^{-3}\) to 1.16 g cm\(^{-3}\) (7). Chrispeels and Greenwood (7) showed that increased ER density in *P. vulgaris* was associated with the accumulation of PHA in the lumen of the ER and that heat shock prevented the export of PHA out of the ER.

There is no evidence in aleurone cells that the increase in density of the ER induced by heat shock is caused by the accumulation of proteins in the ER lumen. Rather, our experiments indicate that less newly synthesized protein (Fig. 5) is associated with the ER of heat-shocked cells. The levels of secretory enzymes, particularly \(\alpha\)-amylase, that are known to be associated with the ER are much reduced in heat-shocked aleurone cells, and pulse-labeling experiments do not show the accumulation of high levels of other newly synthesized proteins in the ER following heat stress (Fig. 5).

Whereas the activities of ER marker enzymes are not reduced, the activity of the Golgi apparatus marker enzyme IDPase is dramatically inhibited by heat shock (Fig. 3). No peak of latent IDPase or detergent-solubilized IDPase (Fig. 3) activity is found in membranes from heat-shocked aleurone tissue. Electron micrographs of heat-shocked aleurone cells show the presence of numerous Golgi, however (3) (M Brodl, personal communication). IDPase could be a protein that is rapidly lost from the Golgi apparatus, either by transport to other compartments or by degradation that may be enhanced by heat shock. From this we infer that elevated temperature also inhibits the synthesis of IDPase or enhances the rate of its turnover, making this enzyme an unreliable marker for the Golgi apparatus in heat-shocked aleurone cells.

We have confirmed the observation of Belanger et al. (3) that aleurone cells synthesize HSPs following exposure to elevated temperature. Our experiments show that these proteins are localized in different compartments of the aleurone cell (Fig. 5). We found that the lower mol wt HSPs of the aleurone layer are associated with the ER and MT/PM fractions of the heat-shocked aleurone cell (Fig. 5). At least eight proteins with Mr between 15,000 and 30,000 (indicated by dots in Fig. 5) are associated with the MT/PM fraction of the density gradient (12). There have been several reports of the localization of low molecular weight HSPs in mitochondria and plastids of plant cells (23). In heat-shocked soybean seedlings proteins with Mr between 20,000 and 25,000 become associated with both mitochondria (17) and plastids (25). Two newly synthesized proteins at approximately 25,000 and 30,000 Mr are found associated with the ER of heat-shocked aleurone cells (Fig. 5), and Cooper and Ho (9) have also localized a Mr 25,000 polypeptide to the ER of heat-shocked corn roots. Because levels of ER-associated HSPs in the aleurone are low, it is unlikely that these proteins contribute significantly to the increased density of the ER following heat shock.

We conclude from our experiments on the effects of heat shock on barley aleurone layers that this stress does not inhibit the synthesis of \(\alpha\)-amylase by causing destruction of ER. To reconcile the ultrastructural observations of Belanger et al. (3) with the data reported in this paper, we suggest that rather than being destroyed by heat shock, the ER is reorganized into a system that is unable to support the synthesis or transport of secretory proteins.

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LITERATURE CITED