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Heat Stress Enhances Phytohemagglutinin Synthesis but Inhibits Its Transport Out of the Endoplasmic Reticulum[1]

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ABSTRACT

In this study we examined the effect of heat stress (up to 6 hours at 43°C) on the biosynthesis and transport of phytohemagglutinin (PHA) in cotyledons of developing seeds of the common bean, *Phaseolus vulgaris*. Heat stress resulted in a decrease of total protein synthesis and an enhancement of the synthesis of heat shock proteins and PHA. Pulse chase experiments showed that a considerable proportion of the newly synthesized PHA was present in the endoplasmic reticulum (ER)/Golgi fraction and did not readily chase-out. Analysis with endoglycosidase H showed that the oligosaccharide sidechains of PHA were almost entirely in the high mannose configuration, indicating that most of the newly synthesized PHA was in the ER. However, some of the PHA became fucosylated at 43°C, indicating fucosyltransferase activity. That the biosynthesis and secretion of fucosyl-containing cell wall polymers proceeded normally at 43°C provided evidence that certain Golgi functions (*i.e.* transport to the cell wall) remained unaffected by heat stress. The ER obtained from these heat stress cotyledons had a greater density (1.16 g·cm^{-3} at 43°C instead of 1.14 g·cm^{-3} at 22°C) in sucrose gradients. Ultrastructural observations showed that the width of the lumen of the ER cisternae had increased from 20 nanometers at 22°C to 60 to 80 nanometers at 43°C; the lumen was filled with electrondense material presumed to be protein. The experiments are interpreted as evidence that heat stress imposes a block in the transport of PHA out of the ER. Whether heat stress affects the ER itself or alters the conformation of PHA, thereby preventing its transport, is not clear.

The response to heat stress of almost all organisms examined to date is characterized by the expression of genes encoding stress proteins (heat shock proteins), and a concomitant decrease in the synthesis of gene products which were being made before heat stress (2, 3, 22). Organisms appear to differ in the extent to which normal protein synthesis is attenuated as well as in the mechanisms responsible for this attenuation. For example, *Drosophila* apparently sequesters constitutively expressed mRNAs from the protein synthesis machinery while yeast rapidly catabolizes these mRNAs during the initial heat stress period (17). Developing plant embryos (seeds) appear to exhibit yet another response to heat stress. In soybean embryos exposed to heat stress, the synthesis of the storage proteins actually increases at the same time as stress proteins are synthesized (18). Mascarenhas and Altschuler (18) also observed that the normal proteolytic processing of the storage proteins was impaired. Because of our interest in the intracellular transport of storage proteins and lectins in developing embryos (8, 9), we have examined the effect of heat stress on the transport of PHA[3] to the protein bodies in the cotyledons of the common bean *Phaseolus vulgaris*. The cotyledons are the main storage organs of the developing plant embryos; they store considerable amounts of protein in special organelles called protein bodies. Our results show that the synthesis of PHA is enhanced as a result of heat stress (43°C), but that the transport out of the ER is greatly reduced. Ultrastructural observations show that the width of the ER lumen increases 3- to 4-fold and that the lumen is filled with electrondense material. These observations are discussed in the framework of the hypothesis that heat stress results in partial denaturation of proteins, exposing hydrophobic regions which interact, thereby causing the proteins to form insoluble aggregates (21). The normal transport processes from the ER to the Golgi may not be able to handle these insoluble aggregates.

MATERIALS AND METHODS

Materials. Plants of *Phaseolus vulgaris* cv Greensleeves were grown in a greenhouse, and midmaturation seeds (200–250 mg/cotyledon) used. L-[^{3}H]leucine (142 Ci/mmol) was purchased from New England Nuclear and D-[2-^{3}H]mannose (15.8 Ci/mmol), D-[6-^{3}H]glucosamine hydrochloride (24.8 Ci/mmol), and L-[5,6-^{3}H]fucose (25.6 Ci/mmol) were purchased from Amersham. Chemicals were from Sigma Chemical Co., unless otherwise noted.

Heat Treatment and Radioactive Labeling. The cotyledons were placed flat-side down on Parafilm in a Petri dish lined with moist filter paper (23) with 10 μl of nutrient medium (23) underneath each cotyledon. The Petri dish was sealed with Parafilm and kept at room temperature (22°C) or in a 43°C incubator for 1 h. Each cotyledon was labeled with 5 μCi of radioactive precursor which was added to the nutrient medium, and kept at room temperature or returned to the incubator. The radioactive tissue was collected, cutting one thin slice from the flat surface of the cotyledon exposed to the radioactive precursor. The remainder of the cotyledon was discarded.

Homogenization and Fractionation and Organelles. The radioactive tissue (4–6 slices) was homogenized in 4 ml of PBS (10 mM K-phosphate, 150 mM NaCl, pH 7.4) and the cell walls and debris removed by centrifugation at 1000*g* for 10 min. To obtain incorporation into the cell wall fraction the pellet was washed four times with PBS containing 1% (v/v) Tween 20 and two times with PBS. To obtain incorporation into proteins an aliquot was precipitated with TCA and filtered on a membrane filter.

For the isolation of intact organelles, the radioactive slices from 4 to 6 cotyledons were homogenized in 100 mM Tris HCl

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[3] Abbreviations: PHA, phytohemagglutinin; endo H, endo-β-*N*-acetylglucosaminidase H.

(pH 7.8) containing 1 mM Na-EDTA and 12% (w/w) sucrose, and the homogenate centrifuged at 1000*g* for 10 min. To obtain a total ER/Golgi fraction the homogenate was loaded on a discontinuous gradient consisting of 1 ml of 35% (w/w) sucrose and 8 ml of 16% (w/w) sucrose with Tris HCl (100 mM, pH 7.8) and EDTA (1 mM). Centrifugation at 150,000*g* (average) for 90 min resulted in an organelle fraction at the 16/35 interface and a soluble fraction (the load portion of the gradient). The soluble fraction contained cytosolic macromolecules as well as the contents of the protein bodies because these large fragile organelles break during the homogenization procedure (11, 12). To fractionate the subcellular organelles the cleared homogenate was first applied to a Sepharose 4B column which separates organelles and vesiculated membranes from the soluble fraction (24), and the organelles subsequently fractionated on a linear 16 to 54% (w/w) sucrose gradient (11). These procedures and those for measuring the ER--marker enzyme NADH-Cyt *c* reductase have all been described (7, 11).

Isolation of PHA. PHA was purified from total homogenates, organelles, or gradient fractions by affinity chromatography using thyroglobulin-Sepharose as described by Felsted *et al.* (13). The isolated PHA was dialyzed against water and freeze dried.

SDS-PAGE and Fluorography. Aliquots of protein fractions were precipitated with 1.5 volumes of methanol and 0.006 volumes of glacial acetic acid and kept at −20°C for 12 h. The protein was washed with 2 ml of 90% (v/v) aqueous acetone at 0°C and subjected to analysis of SDS-PAGE on 12.5% acrylamide gels. Fluorographs were obtained (5) using XAR-5 film (Eastman Kodak, Rochester, NY). Relative molecular mass markers were from Amersham.

Treatment with Endo H. Treatment with endo H (Miles, Elkhart, IN) was carried out for 24 h at 37°C in 50 mM sodium acetate (pH 5.8), with native PHA or with PHA denatured by boiling for 3 min in 1% SDS. Sufficient BSA was added to achieve a ratio of SDS to protein of 1.2 before the addition of endo H.

Electron Microscopy. Developing cotyledons were excised and incubated on nutrient medium (19) for 4 h at either 22 or 43°C. Following incubation, the cotyledons were immediately immersed in 3% glutaraldehyde, 2% acrolein in 25 mM k-phosphate buffer (pH 7.2), 0.5 M sucrose. The cotyledons were sliced 1 to 2 mm thick under the fixative and the slices fixed for 2 h at room temperature followed by 4 h at 4°C with gentle agitation. Following two 8 h washes with cold buffer, the cotyledon slices were postfixed for 4 h at 4°C with 2% OsO_4 in the same buffer. The tissues were rinsed with distilled H_2O (four changes over 16 h at 4°C) and dehydrated using 50, 70, 80, 90, and 95% ethanol (2 h each step). The sections were infiltrated with a 1:1 mixture of 95% ethanol:L.R. White resin, followed by three incubations in 100% L.R. White, 4 h for each step. Following polymerization of the resin for 8 h at 60°C, sections of embedded tissues were cut 50 to 70 nm thick, and mounted on Formvar coated grids. Staining was done with saturated uranyl acetate in 50% ethanol followed by Reynold's lead citrate. The sections were examined using a JEOL 100 S electron microscope.

RESULTS

Heat Stress Enhances the Biosynthesis of PHA. The effect of different temperatures on the incorporation of [^{3}H]leucine into cellular proteins and PHA of isolated cotyledons is shown in Figure 1. Incorporation was allowed to proceed for 2.5 h at the same temperature as the 90 min pretreatment. The results show that incorporation of [^{3}H]leucine into cellular proteins and PHA increased up to 37°C. At 43°C there was a drop in the incorporation of [^{3}H]leucine into proteins in general, but an increase in the incorporation into affinity-purified PHA. As a result there was a doubling in the percentage of radioactive [^{3}H]leucine incorporated into PHA at 43°C compared to room temperature. The greater incorporation of [^{3}H]leucine into PHA was confirmed by fluorography of the polypeptides separated by SDS-PAGE (Fig. 2). The synthesis of both the E and L polypeptides of PHA was enhanced at 43°C (double arrow). The fluorograph shows that there is a small increase in the M_r of PHA at 43°C,

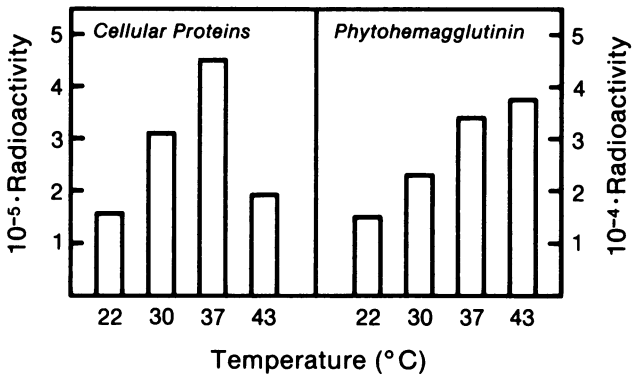

FIG. 1. Effect of heat stress on the incorporation of [^{3}H]leucine into total cellular proteins and PHA. Cotyledons were given a 90 min pretreatment at the temperature (°C) indicated, then allowed to incorporate [^{3}H]leucine for 2.5 h at these temperatures. PHA was isolated by affinity chromatography.

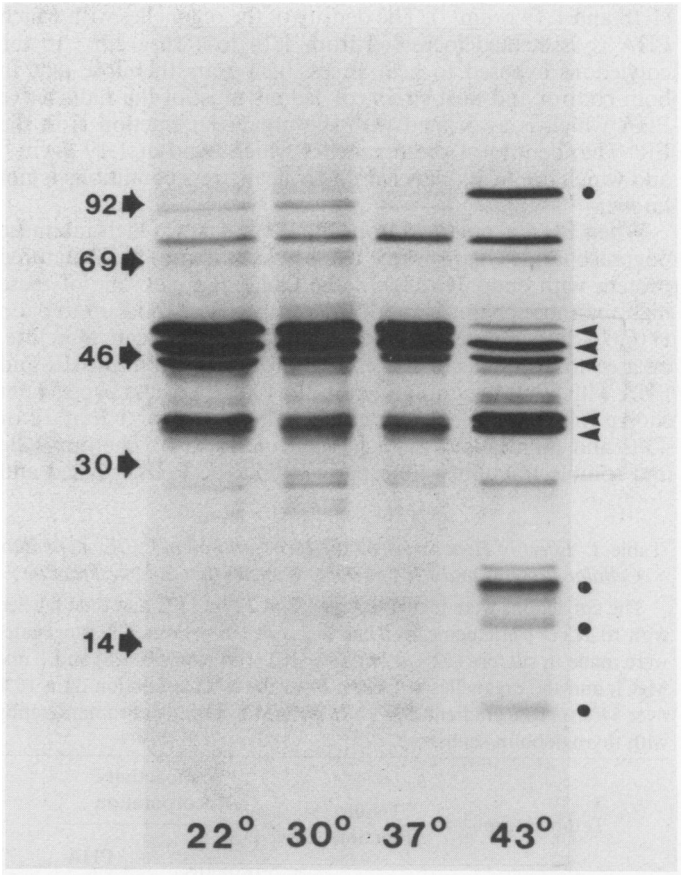

FIG. 2. Analysis by SDS-PAGE and fluorography of the polypeptides synthesized at different temperatures. Same conditions as in Figure 1. The gel was loaded with equivalent amounts of tissue extract in each lane. Double arrow indicates PHA, triple arrow indicates phaseolin, and solid circles indicate heat shock proteins. Numbers on the left show the M_r of standard proteins ($M_r \cdot 10^{-3}$).

due to a lack of processing of the high mannose oligosaccharide chains (see below). At the elevated temperature, several heat-shock proteins were synthesized in the tissue (asterisks), while the biosynthesis of phaseolin (triple-arrow) was decreased, especially at the highest temperature. Heat stress differentially affected the synthesis of phaseolin polypeptides; synthesis of the largest polypeptide was more inhibited than the others. To determine in which subcellular fraction the newly synthesized PHA was located, cotyledons were labeled for 5 h after a 1 h pretreatment, and the radioactive tissue homogenized and fractionated on discontinuous sucrose gradients into organelles (mostly ER, Golgi, and mitochondria) and soluble fractions. Under these conditions of homogenization and centrifugation the protein bodies rupture and release their contents into the cytosolic fraction. The results (Table I) showed that heat stress caused a 2.5-fold increase in radioactive PHA in the organelle fraction.

Heat Stress Causes the Accumulation of Unprocessed PHA in the ER. PHA is synthesized on the RER, and its transport to the protein bodies is mediated by the Golgi complex (7). The organelle fractions of cotyledons labeled for 3h with [^{3}H]leucine at 22 and 43°C were analyzed on isopycnic sucrose gradients to determine the density of the organelles with which the newly synthesized PHA was associated. The results, presented in Figure 3, show that at 22°C the organelles which contained most of the incorporated radioactivity had a density similar to the ER-marker enzyme NADH-Cyt *c* reductase (see also Fig. 1 in Ref. 10). Heat stress caused a shift in the density of the marker enzyme NADH-Cyt *c* from 1.14 g·cm^{-3} to 1.16 g·cm^{-3} and the appearance of a double peak of incorporation into total proteins (1.16 and 1.19 g·cm^{-3}). The density of the organelles with which PHA is associated increased from 1.14 to 1.16 g·cm^{-3} in the cotyledons exposed to heat stress. It appears therefore that in both control and heat-stress cotyledons most of the radioactive PHA which is associated with the organellar fraction is in the ER. The identity of the organelles which band at 1.19 g·cm^{-3} and which are highly labeled under heat-stress conditions is not known.

When PHA is obtained from the ER it has two high-mannose oligosaccharide chains which can be cleaved from the denatured protein with endo H (25). In the Golgi complex one of these high-mannose chains is modified and becomes resistant to endo H (6, 7). Susceptibility of the sidechains to endo H can, therefore, be used to distinguish between PHA which is still in the ER and PHA which has been modified in the Golgi. For the experiment shown in Figure 4, cotyledons were pretreated for 1 h at 22 or 43°C and then labeled with [^{3}H]leucine for 5 h. The organelle and soluble fraction were separated and the PHA isolated and denatured by boiling with SDS. The radioactive PHA was digested with endo H, and the products analyzed by SDS-PAGE and fluorography (Fig. 4). Lane 1 in this figure shows total PHA (*i.e.* mostly PHA from the protein bodies) from cotyledons labeled in the presence of tunicamycin. The fastest-running doublet (lowest M_r) represents unglycosylated PHA, while the upper, incompletely resolved doublet represents glycosylated PHA. PHA from the organelles of control and heat stress cotyledons is shown in lanes 2 and 4. PHA from organelles always has a slightly greater M_r than total PHA (most of which comes from protein bodies) because of the processing of one of the two high mannose oligosaccharide chains (25). When PHA from the organelles of the control cotyledons (22°C) was treated with endo H we obtained two new doublets (lane 3). The upper doublet (arrow 2) represents PHA which has one endo H-resistant and one endo H-susceptible oligosaccharide (PHA from the Golgi complex), and the lower doublet (arrow 3) represents PHA which has two endo H susceptible oligosaccharides (PHA from the ER). It occupies the same position on the gel as unglycosylated PHA synthesized in the presence of tunicamycin (lane 1, and Ref. 25). When PHA from the organelles of the heat-stressed cotyledons was treated with endo H, most of the radioactivity was in the lower doublet (lane 5) indicating that most of the PHA had two high-mannose oligosaccharides susceptible to endo H. These results confirm that heat stress causes an accumulation of PHA

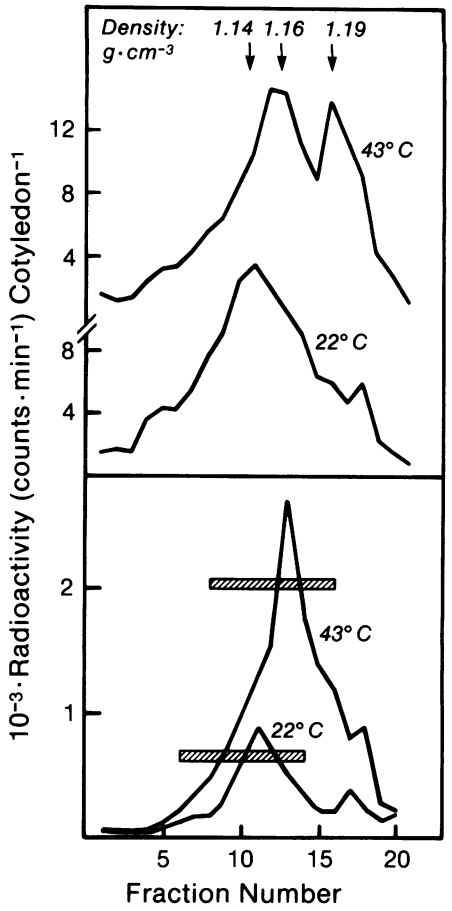

FIG. 3. Analysis of organelles on isopycnic sucrose gradients. Cotyledons were labeled with [^{3}H]leucine at 22 or 43°C and the homogenate fractionated on Sepharose 4B to obtain organelles. The organelles were separated on linear (16–48%) sucrose gradients on a cushion of 54% sucrose. Each fraction was analyzed for incorporation into total protein (upper panel) and incorporation into PHA (lower panel) as well as for the activity of NADH-Cyt *c* reductase (shown as horizontal hatched bars).

Table I. *Effect of Heat Stress on the Incorporation of [^{3}H]Leucine into Cellular Proteins and PHA in the Organelles and Soluble Fraction*

The cotyledons were incubated for 1 h at 22 or 43°C and then labeled with 10 μCi of [^{3}H]leucine for 5 h at the same temperature. Homogenates were made in sucrose (12% w/w) Tris HCl (100 mM, pH 7.8) and 1 mM $MgCl_2$ and the organelles separated from the soluble fraction on a 16% over 54% sucrose gradient. PHA was isolated by affinity chromatography with thyroglobulin-sepharose.

Temperature	Tissue Fraction	Radioactivity Incorporation	
		Cellular proteins	PHA
°C		*cpm·cotyledon*$^{-1}$	
22	Organelles	348,460	11,655
	Soluble	351,000	16,170
43	Organelles	303,240	29,540
	Soluble	188,900	12,200

with 2 high-mannose oligosaccharides. Either this PHA is mostly in the ER, or it has reached the Golgi, but Golgi-modifying enzymes failed to act on it.

The Effect of Heat Stress on Golgi Functions. The results presented above indicate that there is an increase in ER-associated PHA, but leave open the possibility that heat stress impairs various Golgi functions. Golgi function under heat stress (43°C) was evaluated by measuring the incorporation for [^{3}H]Man, [^{3}H]GlcN, and [^{3}H]Fuc into cytoplasmic and cell wall associated molecules and comparing it to incorporation at 22°C (Table II). The results show that at 43°C incorporation of sugars was greater than at 22°C, and that newly synthesized molecules became incorporated into the cell wall. Heat stress therefore does not inactivate some glycosyl transferases, or block the transport of secretory vesicles to the cell wall. The increased incorporation of sugars at 43°C could in each case be accounted for by a similar increase in the uptake of the precursor into the tissue (data not shown).

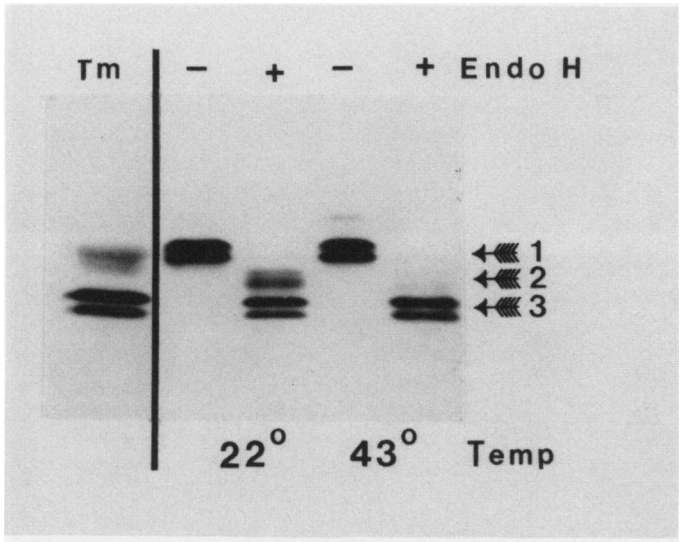

FIG. 4. Susceptibility of the sidechains of PHA to removal by endoglycosidase H. Cotyledons were labeled with [^{3}H]leucine for 5 h after a 1 h pretreatment, either at 22 or at 43°. The organelle fraction was obtained from the homogenates on a discontinuous sucrose gradient, and PHA isolated by affinity chromatography. The PHA was denatured by boiling with SDS, digested with endo H, and the polypeptides analyzed by SDS-PAGE and fluorography. The lane on the left shows the position of PHA and of the unglycosylated polypeptides of PHA synthesized in the presence of tunicamycin. Arrows on the right indicate the position of fully glycosylated PHA (1), PHA which has lost one high mannose oligosaccharide chain (2), or 2 oligosaccharide chains (3). Treatments with and without endo H and temperature (temp) are indicated on the figure.

In an experiment similar to the one shown in Table II, cotyledons were labeled with [^{3}H]Fuc, and the tissue homogenate fractionated into organelles and soluble fractions. The incorporation of [^{3}H]Fuc into PHA was determined and the results showed that at 43°C there is a 3-fold increase over the value at 22°C in the incorporation of [^{3}H]Fuc into PHA associated with the membranous organelles. This increase is similar to the increase in [^{3}H]Fuc taken up by the cotyledons as a result of heat stress. This result shows that PHA which reaches the Golgi is fucosylated there.

Heat Stress Causes an Inhibition of Transport of PHA and an Accumulation in the ER/Golgi Fraction. Pulse-chase experiments were carried out to determine if the apparent accumulation of radioactive PHA in the organelle fraction was due to an inhibition of transport. Cotyledons were pretreated at 22 or 43°C for 60 min, then incubated with [^{3}H]leucine for 90 min, the excess radioactivity subsequently removed and the incorporated radioactivity chased with nutrient medium. Pretreatment, pulse, and chase were all done either at 22 or at 43°C. The organelle fraction (ER + Golgi) and soluble fraction were isolated with discontinuous sucrose gradients at different times thereafter, and PHA purified from each fraction by affinity chromatography. Figure 5 shows the time course of the chase-out of radioactivity from PHA in the ER/Golgi fraction for the control and heat stressed material, as well as the appearance of radioactivity in PHA in

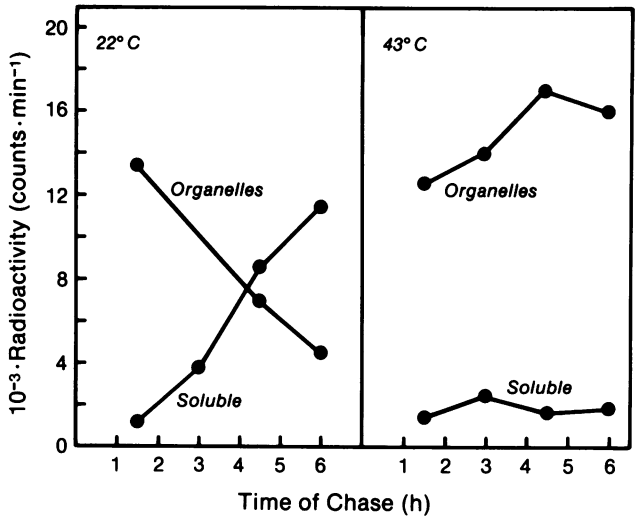

FIG. 5. Pulse-chase labeling of PHA with [^{3}H]leucine under control and heat stress conditions. Cotyledons were pretreated for 60 min, labeled for 90 min with [^{3}H]leucine, then chased with nutrient medium for the times indicated. Homogenates were fractionated on discontinuous gradients in organelles (ER/Golgi) and soluble (cytosol + protein body content) fractions and PHA isolated from each. Pretreatment, labeling, and chase were done either at 22 or at 43°C.

Table II. *Incorporation of [^{3}H]Man, [^{3}H]GlcN, and [^{3}H]Fuc into Cytoplasmic and Cell Wall Associated Macromolecules*

Cotyledons were pretreated for 1 h at 22 or 43°C and then labeled at these temperatures for 5 h with the radioactive precursors in the presence of nutrient medium.

Temperature	Tissue Fraction	[^{3}H]Man	[^{3}H]Fuc	[^{3}H]GlcN
°C			*cpm · cotyledon*$^{-1}$	
22	Cell Wall	18,200	64,300	1,220
43	Cell Wall	26,400	79,800	1,340
22	Cytoplasm	76,000	44,000	32,000
43	Cytoplasm	48,300	111,300	64,900

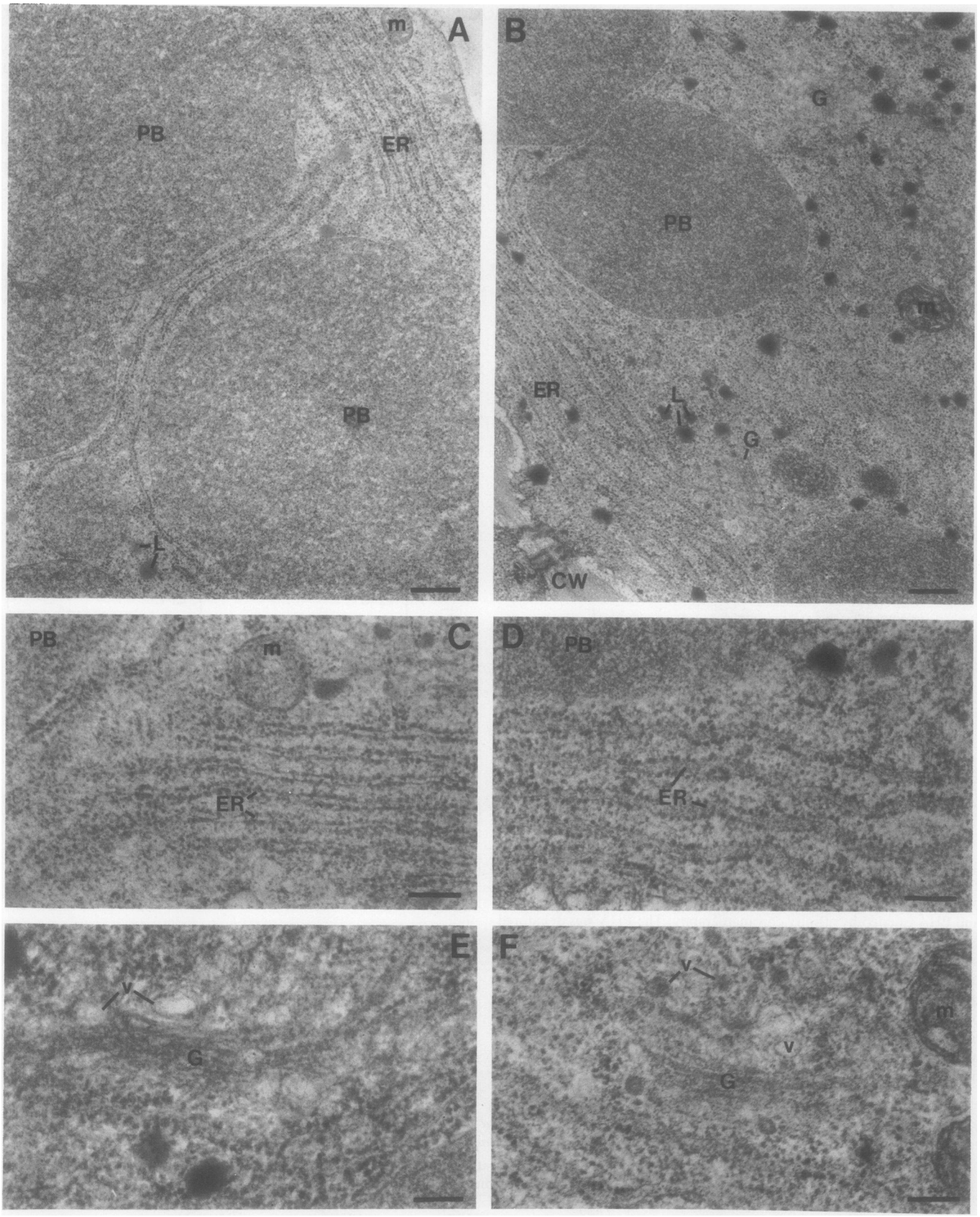

FIG. 6. Electron micrographs of storage parenchyma cells from developing cotyledons incubated at either 22°C (A, C, E) or 43°C (B, D, F) for 4 h. A and C, Low magnification micrographs illustrating the general structure of the storage parenchyma cells at 22°C (A) and 43°C (B). Cells in both instances were typified by the presence of numerous protein bodies, small lipid bodies, and stacks of ER cisternae. Golgi complexes though not shown in Figure 6A, were present and numerous under both conditions. Note particularly the dilation of the ER cisternae when cotyledons were subjected to the 43°C incubation (B). Both A and B: ×18,000, bar equals 500 nm. C and D, Detail illustrating the RER cisternae in storage parenchyma cells from cotyledons incubated at either 23°C (C) or 43°C (D). Note the dilation of the ER cisternae in the 43°C incubated sample (D), and the accumulation of electron dense material, probably protein, within the ER cisternae at this temperature. Intermembranal width of ER cisternae was approximately 20 nm at 22°C (C) compared to 60 to 80 nm at 43°C (D). E and F, No change in the frequency or appearance of the Golgi due to incubation at 43°C (F) was evident when compared to the 22°C control (E). E, ×64,000, bar equals 150 nm; F, ×40,000, bar equals 250 nm. CW, cell wall; ER, rough endoplasmic reticulum; G, Golgi apparatus; L, lipid body; m, mitochondrion; PB, protein body; v, Golgi vesicle.

the soluble fractions. With these conditions of homogenization and fractionation the soluble fraction also contains the content of the protein bodies. The results show that heat stress causes a very strong inhibition of the chase-out of radioactivity in PHA in the organelle fraction, and a concomitant inhibition of the appearance of radioactive PHA in the soluble fraction. In a subsequent experiment we determined that the inhibition of transport cannot be reversed by returning the temperature to 22°C during the chase (data not shown). Thus, there is not a rapid recovery of intracellular transport after heat stress.

Changes in Ultrastructure Resulting from Heat Stress. Changes in the ultrastructure of the ER and Golgi as a result of heat stress are shown in Figure 6. Low magnification micrographs show the presence of protein bodies, RER cisternae, lipid bodies and Golgi complexes in cells at 22°C (Fig. 6A) and 43°C (Fig. 6B). The cisternae of the ER were dilated at 43°C, as is evident by comparing Figure 6, C and D. Incubation at 43°C caused an increase in the width of the lumen of the ER from 20 nm to 60 to 80 nm. The cisternae appear to be filled with an electrondense material, presumably protein that is not being transported out of the ER. Examples of Golgi structures are shown in Figure 6, E and D. No differences were apparent in Golgi structure or Golgi vesicles between the 22 and 43°C samples.

DISCUSSION

The results presented in this paper demonstrate that heat stressed bean cotyledons synthesize more PHA than cotyledons kept at 22°C, but fail to transport this PHA to the protein bodies. Our results indicate that the major block along the transport pathway is probably between the ER and the Golgi. This conclusion is based on the following observations. First, the ER from heat stressed cotyledons has a greater density on sucrose gradients, has a much larger lumen, and contains electron-dense material. Second, PHA associated with the total ER/Golgi fraction has oligosaccharides which are mostly of the high-mannose type indicating that this PHA has not yet been modified in the Golgi. Third, because heat stress does not affect the fucosylation of the PHA which reaches the Golgi, or the biosynthesis and transport of polysaccharides to the cell wall, certain Golgi functions appear to be unaffected by heat stress. We cannot rule out the possibility that heat stress affects the transport of vesicles from the Golgi to the protein bodies without inhibiting the transport of vesicles to the cell wall. The results obtained here help explain findings of Mascarenhas and Altschuler (18) who observed that the soybean storage protein glycinin failed to be proteolytically processed under heat stress conditions. Proteolytic processing of storage proteins has been shown to occur in the protein bodies (12), and a lack of processing of the soybean storage protein probably indicates an inhibition of transport. The inhibition of processing was observed whether the heat stress was imposed suddenly (18) or gradually (1) indicating that the inhibition of transport occurs whether heat stress is applied gradually or suddenly.

It is not clear whether the heat stress temperature has a negative effect on the ER itself resulting in an inhibition of transport, or whether the higher temperature causes a conformational change in the protein (PHA) thereby preventing its transport out of the ER. Such a conformational change could cause the protein to aggregate, prevent it from forming tetramers, and/or result in poor recognition by a putative transport receptor. Pelham (21) recently proposed that heat stress causes a partial denaturation of many proteins, thereby exposing hydrophobic regions which cause the proteins to interact and form insoluble aggregates. The function of heat shock proteins could be to interact with the partially denatured proteins and to prevent the aggregation. Circumstantial evidence indicates that the ER of mammalian cells contains a heat shock-like protein which may be involved in protein folding and assembly of oligomers (20). Heat stress may overload this system causing improper folding, improper oligomer formation and/or aggregation. Recent evidence shows that proper folding (14) and oligomerization of monomers (16) are prerequisites for intracellular transport. Experiments on the transport of G-protein of Vesicular Stomatitis Virus show that changes which decrease the solubility of the protein at slightly elevated temperatures (38°C *versus* 30°C) and cause aggregation *in vitro* also impair transport of the protein from the ER to the cell surface (15).

In soybean seeds the biosynthesis of both storage proteins, glycinin and conglycinin, is enhanced under heat stress conditions (1, 18). This does not appear to be the case in *Phaseolus vulgaris*. The synthesis of phaseolin was inhibited at the same time as the synthesis of PHA was enhanced. Although we made no specific measurements, the results obtained with SDS-PAGE (Fig. 2) indicate that the synthesis of phaseolin may decline less rapidly than that of other proteins as the temperature is raised. Altschuler and Mascarenhas (1) proposed that this phenomenon may have adaptive value, allowing the developing seeds to continue synthesizing reserves under heat stress conditions. However, synthesis is of no avail to the plant unless the products are also transported and stored in protein bodies. It would therefore be interesting to know whether and when the transport of these proteins resumes after the heat stress is lifted. The *in vitro* system used here, *i.e.* isolated cotyledons on nutrient medium, is probably not well suited to investigate this question, since we did not observe any resumption of transport after returning the cotyledons for several hours at 22°C. A system in which intact plants or cultured pods are subjected to heat stress would be better suited to investigate the resumption of protein transport.

Our results on the effect of heat stress on the ER contrast sharply with those of Belanger *et al.* (4) who studied the effect of heat stress (40°C) on the ER-mediated secretion of α-amylase by barley aleurone cells. They observed a total inhibition of α-amylase secretion coupled with the complete destruction and vesiculation of the ER cisternae. This effect of heat stress was accompanied by the rapid decay of α-amylase mRNA. In contrast, the increased synthesis of PHA is probably dependent on an increase in PHA mRNA as has been shown for the mRNAs of soybean storage proteins in soybean cotyledons subjected to heat stress (1, 18). The different responses to heat stress of cotyledons of developing seeds on the one hand and aleurone cells of young seedlings on the other, may well reside in the physiological nature of the processes. An interruption of α-amylase synthesis and rapid catabolism of stored reserves may have adaptive value for a small seedling, while a continuation of storage protein synthesis may have adaptive value for the developing seed.

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