Heat Shock Proteins and Protection of Proliferation and Translation in Mammalian Cells

James J. Sciandra and John R. Subjeck

Division of Radiation Biology, Roswell Park Memorial Institute, Buffalo, New York 14263

ABSTRACT

The characteristics of heat shock protein(s) (HSP) and thermotolerance induction were examined in Chinese hamster ovary cells following two diverse nonlethal heat shocks: a continuous 41°C exposure and a 45°C, 5-min exposure. While induction of both HSP and thermotolerance were observed to initially develop simultaneously, a significant period was observed during which HSP induction was not accompanied by any further increase in the cells' ability to survive a thermal stress. Conversely, the achievement of the full tolerant state, as measured by colony survival, correlates in these instances with the moment at which development of the induction of HSP either ceased or began to be repressed. When a 99% lethal 45°C, 22-min heat shock was examined, HSP and a thermal resistance were again observed to develop synchronously, despite the fact that the tolerance was measured in only 1% of the cell population. In this instance, a new protein of molecular weight 66,000 was observed which was not visibly induced by either of the two nonlethal treatments. Finally, the ability of heat-shocked thermotolerant cells to translate proteins following a second heat challenge (protection of translation) was investigated as an alternative measure of thermotolerance. The phenomenon paralleled the induction and repression phases of HSP synthesis and was therefore not related to thermotolerance.

INTRODUCTION

A hyperthermia pretreatment can confer a significant protective effect to a later heat treatment. This induced thermotolerance has been well studied in mammalian cells using a colony survival assay (1) and has been found to correlate generally with the induction of HSP (2, 5, 7-9, 13, 14). However, more recent reports have shown that, despite the general correlation between the expression of HSP and thermotolerance, specific violations of the relationship can occur (6, 15). We examine here in further detail the relationship between HSP synthesis and thermotolerance (as determined by colony-forming ability) following 2 diverse nonlethal heat shocks and demonstrate that the correlation between these phenomena is not stringent. We also report that the 2 major HSP in the M, 70,000 region are differentially expressed as a function of the severity of the heat treatment. Finally, the ability of heat-shocked thermotolerant cells to translate proteins following a second heat challenge (protection of translation) is investigated as an alternative measure of thermotolerance.

MATERIALS AND METHODS

Chinese hamster ovary fibroblasts obtained from Los Alamos National Laboratory were maintained as monolayer cultures at 37°C in Ham's F-10 medium (Grand Island Biological Co.) supplemented with 15% newborn calf serum (cycle time, 12.5 hr). All experimentation was performed in T-25 flasks (Corning Glass Works) using asynchronous log-phase cells. Prior to heating, medium was exchanged with complete F-10 medium and allowed to equilibrate to incubator conditions. Flasks were immersed horizontally into a constant-temperature water bath (Haake FK-2) for various times at 45°C ± 0.1°C. Cells exposed to 41°C were immersed in the water bath for 10 min (41°C) and then transferred to a humidified, pH-controlled incubator set at 41°C for various times. At various times following heating, cells were either trypsinized and plated for single cell survival or labeled for 1 hr with [35S]methionine (Amersham/Searle Corp., 1400 Ci/mmol) as discussed elsewhere (14).

Following labeling, cells were washed twice in F-10 without serum at 4°C, resuspended in Hanks' balanced salt solution with 1 mM tosyl-L-arginine methyl ester (Sigma Chemical Co.), 5 mM EDTA (Sigma), and 1 mM phenylmethylsulfonyl fluoride (Sigma) and sonicated at 4°C. Protein determinations were made on lysate, which was then precipitated with trichloroacetic acid (Fisher Scientific Co.). The protein synthesis rate at time ti was determined by adding the label between ti-1/2 and ti + 1/2 hr. In each experiment, an equal mass of protein was loaded in each gel lane for single cell survival or labeled for 1 hr with [35S]methionine (Amersham/Searle Corp.). Densitometric determinations were made on lysate, which was then precipitated with trichloroacetic acid (Fisher Scientific Co.) and dissolved in a sample buffer containing 1% sodium dodecyl sulfate (Accurate Chemicals and Science Corp.), 125 mM Tris-chloride (pH 6.8), 10% glycerol, 1% 2-mercaptoethanol, 1 mM EDTA, and 0.004% bromophenol blue. Culture tubes containing specimens were suspended in boiling water for 2 min. SDS-polyacrylamide gel electrophoresis utilized the discontinuous, Tris-glycine system of Laemmli (4) as modified and described (3). Coomassie blue-stained gels were photographed prior to being dried onto filter paper for autoradiography. In all radiograms shown in this paper, a 24-hr exposure of the dried gel to Kodak XAR-5 film was used. In the quantitative studies, specific proteins were located using Coomassie Brilliant Blue and were cut with a razor blade from the gel. Labeled protein was then extracted overnight in a 0.2% SDS-phosphate buffer as described previously (14). The protein synthesis rate at time ti was determined by adding the label between ti-1/2 and ti + 1/2 hr. In each experiment, an equal mass of protein was loaded in each gel lane using the Bio-Rad protein determination assay. Various molecular weights assigned to proteins were estimated based on comparison with Bio-Rad molecular weight standards. Tropomyosin was identified using a purified preparation from avian pectorals muscle. Analysis of protein patterns of heat-challenged thermotolerant cells labeled with [35S]methionine was just as described. In these studies, pretreated cells (45°C/5 min) were challenged at 45°C for 12 min at various times after the 5-min treatment and were labeled immediately following the second heat shock. In some instances, scans of Coomassie blue-stained gels were made using a gel scanner (Quick Scan Electrophoresis Dosimeter; Helena Laboratories) to estimate the total amount of individual proteins. The relative percentage of a specific protein was determined from the area under the peak with a correction for background density.

RESULTS

Chart 1 shows a cell survival study in which cells are heat-shocked at 45°C for 5 min at Time 0 and then incubated for variable times, following which a second more severe heat
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Chart 1. A 45°, 32-min exposure is delivered to Chinese hamster ovary cells as a continuous treatment or as a 5-min treatment at Time 0 separated by incubation for the hours indicated at 37° from a later 27-min treatment. The effect of a 45°, 27-min treatment alone is indicated on the Y-axis. Bars, S.E.

Treatment (45°, 27 min) is applied to measure thermotolerance. The initial shock itself results in no cell death. As the time of separation of the 2 treatments increases to 4 hr, the cells become increasingly resistant to the 27-min treatment. At later times, the conferred tolerance promptly levels off. Fig. 1 shows a gel electrophoretic study following the same 45°, 5-min heat shock. Fig. 1a shows a Coomassie blue-stained gel, and Fig. 1b is the autoradiogram of Fig. 1a. The Coomassie Blue-stained gel reflects the total protein present, while the autoradiogram indicates the rate of protein synthesis at each time point. The induction of HSP in Fig. 1b begins to become evident at 1 hr and increases to a maximum level at 4 hr. The induction continues to approximately 12 hr post-shock. This data is presented in quantitative form in Chart 2. While this comparative study shows that HSP induction and thermotolerance correlate well during the induction phase, HSP synthesis continues at induced levels (at 4 to 12 hr; Chart 2) at a time when no additional change in tolerance occurs. Conversely, the precise time at which tolerance levels off predicts the time at which the heat shock response is curtailed and begins to be slowly repressed. Gel scans of 3 of the lanes from Fig. 1a (control, 4 hr, and 8 hr) are presented in Chart 3. Although thermotolerance plateaus at 4 hr, all 3 of the heat shock proteins indicated continue to accumulate to 8 hr (and 12 hr; not shown), in agreement with the pulse-label data. This increase is most clearly seen with HSP 68, where an approximate accumulation in this protein of 1.6-fold (relative to the control) observed at 4 hr is seen to increase to 2.0-fold at 8 and 12 hr post-shock.

Chart 4 shows a survival study in which cells are heated for increasing times at 41° and immediately challenged by a 45°, 27-min treatment to measure thermotolerance. A 1-hr pretreatment (45°, 27 min) confers a 40-fold factor of protection to the 27-min challenge and 1 additional hr (2 hr) results in additional tolerance. Tolerance thereafter levels off. In Fig. 2, a 1-hr pulse-label is applied (at 37°) following the indicated times at 41°. In this case (Fig. 2b), a near-full induction of HSP 89 and HSP 68 is visible at 1 hr, but HSP 110 induction is seen only faintly. Induction

Chart 2. Incorporation of [35S]methionine determined with a scintillation spectrophotometer (and normalized to control levels of protein synthesis) for experiment shown in Fig. 1. HSP 68; A, HSP 89; C, HSP 110. Bars, S.E. for 4 to 6 experiments. — — , HSP 110; — — , approximation of both HSP 68 and HSP 89, which behave in a similar manner. Curves are fitted by eye. At 0-, 1-, and 20-hr points, circles coincide with triangles and are not shown.
Chart 3. Scans of Coomassie Brilliant Blue-stained gel shown in Fig. 1a. Control (a), 4-hr (b), and 8-hr (c) lanes are shown, and the HSP (68, 89, and 110) and actin (A) are indicated. The region of each gel between actin and HSP 110 is shown.

Chart 4. Chinese hamster ovary cells are heated at 41° for the hours indicated, followed by an immediate challenge of 45°, 27 min. Bars, S.E.

Chart 5. Incorporation of [35S]methionine determined with a scintillation spectrophotometer (and normalized to control synthesis levels) for experiment shown in Fig. 2. ●, HSP 68; □, HSP 89; ○, HSP 110. Bars, S.E. for 4 to 6 experiments.

Chart 6 shows a survival study in which cells receive a 45°, 22-min heat treatment. The cells are then returned to 37° and incubated for the indicated times, following which they are challenged by a second treatment of 45° for 10 min to measure thermotolerance. In control cells, this treatment results in a 42% surviving fraction. Unlike the 2 experiments considered above in which the initial heating caused no cellular lethality, the initial treatment applied here kills 99% of the cell population as assayed by colony survival. Survival begins to recover immediately and increases rapidly to approximately 10 to 14 hr post-heat shock. At these times, survival approaches a value which results from the application of the initial treatment alone, and the challenge treatment has no significant effect. Fig. 3 shows the autoradiogram which results after applying a [35S]methionine pulse at increasing times following the initial 22-min heat shock (autoradiogram only). This severe heat treatment leads to a significant reduction in total cell protein synthesis, and the normal level of protein synthesis is never totally restored (e.g., incorporated label into actin = 60 to 70% of control at 20 to 24 hr). The major HSP become evident in the 8- to 12-hr period and peak at 16 to 20 hr. At this time, the level of synthesis HSP 68 is approximately 1.7 times control values, while HSP 89 and 110 are less strongly induced. An important point in this study is the appearance of a HSP at M, 66,000 (HSP 66) just below the major HSP at M, 68,000, observed in the nonlethal heat shocks reported here. HSP 66 was not observed in the 2 preceding nonlethal studies and was not visibly synthesized in unheated, control Chinese cell cultures.
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In the case of the near totally lethal 45°, 22-min inducing heat shock, protein synthesis patterns are indicative of cells which are ultimately doomed (non-colony-forming). It is of interest in this case that HSP induction and recovery of protein synthesis still occurs in a manner which generally correlates with the expression of heat resistance. The expression of thermal resistance in this case might be partially accounted for by a recovery
from hyperthermic sublethal damage. However, the importance of this distinction is unclear, since Henle and Leeper (2) have shown in Chinese hamster ovary cells exposed to a similar heat exposure (17.5 min at 45°) that thermotolerance is reflected by a change in both the slope and the shoulder of survival curves prepared at subsequent times. While the maximum induction of HSP 68 obtained by this severe exposure is comparable to its peak induction level following the 45°, 5-min treatment (and greater than that subsequent to the 41° exposure), an important point with regard to this treatment is the expression of HSP 66 (observed just below HSP 68; Fig. 3). This protein was not visibly induced subsequent to the 45°, 5-min or the 41° treatment by either by 1-dimensional (Figs. 1 and 2) or 2-dimensional gels (data not shown). This suggests that the induction of this protein may be triggered by a more severe inducing treatment relative to HSP 66. We have observed independently that HSP 66 is strongly induced in a histone H1 phosphorylation deficient-temperature sensitive FM3A mutant (10), which is highly thermosensitive, but it is not expressed in the parent FM3A line, which is resistant to the same heat dose.4

While it is still highly probable that HSP play a central role in thermotolerance, the connection may be more complex than the simple dose-effect relationship which we had envisioned previously (14), and it is evident that, in some cases, at least, an overexpression of HSP can occur. In addition, the study presented here has brought to light 2 other important aspects of cellular response to heat: (a) the phenomena of protection of translation; and (b) the differential induction of HSP 66 (relative to HSP 68) following increasingly severe heat exposures. These latter observations suggest areas for further investigation.

**REFERENCES**


7. Landry, J., Chretien, P., Bernier, D., Nicole, L. M. Marceau, N., and Tanguay, 


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Fig. 1. Chinese hamster ovary cells received a 45°, 5-min exposure at Time 0 and were then pulse-labeled with [³⁵S]methionine after incubation at 37° for the hours indicated. Pulse applied between t - ½ to t + ½ hr for each time t. a, Coomassie Blue-stained gel; b, corresponding autoradiogram of a. Left margin, molecular weight standards (top to bottom): phosphorylase b (M, 92,500), bovine serum albumin (M, 66,200), ovalbumin (M, 43,000), and carbonic anhydrase (M, 31,000). Positions of HSP, actin (A), and tropomyosin (T) are indicated (right margin).

Fig. 2. Chinese hamster ovary cells incubated at 41° for the hours indicated followed by a 1-hr [³⁵S]methionine pulse at 37°. a, stained gel; b, corresponding autoradiogram. Molecular weight standards (left) and analyzed proteins (right) are indicated (as in Fig. 1).
Fig. 3. Chinese hamster ovary cells receive a 45°, 22-min exposure at Time 0 and were then pulse-labeled with [35S]methionine after incubation at 37° for the hours indicated (autoradiogram shown). Molecular weight standards (left) and some relevant proteins (right) are indicated (as in Fig. 1).

Fig. 4. Chinese hamster ovary cells are shocked at time 0 at 45° for 5 min. After the hours indicated, cells are heat-shocked a second time at 45° for 12 min. A 1-hr [35S]methionine pulse is then applied immediately. The application of the 45°, 12-min shock to nonpretreated cells is shown (Lane 2/). a, stained gel; b, corresponding autoradiogram. Molecular weight standards (left margin) and some relevant proteins (right margin) are indicated (as in Fig. 1).
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