Modulation of Diamide Toxicity in Thermotolerant Cells by Inhibition of Protein Synthesis

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ABSTRACT

Chinese hamster ovary cells were exposed in vitro to various concentrations of diamide for 1 h at 37°C. This treatment resulted in a dose dependent increase in cytotoxicity. Cells were also heated at 43°C for 15 min, incubated at 37°C for 3 h, and then exposed to various concentrations of diamide. This heat shock has been shown previously to trigger the synthesis of heat shock proteins and the development of thermotolerance. Further, under these experimental conditions both were inhibited if protein synthesis was inhibited by exposure to cycloheximide (M. L. Freeman et al., Cancer Res., 47: 7033-7037, 1988). Diamide toxicity was diminished in cells made thermotolerant by the 43°C/15-min heat shock. For example, at the highest dose used, 0.8 mM, survival increased from 0.93% to 6.1%. However, diamide toxicity was unaffected if the cells were exposed to diamide 3 h after a 43°C/60 min heat shock. This latter heat shock produced significant inhibition of protein synthesis whereas the 15-min heat shock did not (M. L. Freeman et al., Cancer Res., 48: 7033-7037, 1988). Further, a 43°C/15-min heat shock did not confer protection against diamide toxicity if the cells were simultaneously exposed to cycloheximide.

Exposure to 0.8 mM diamide was shown to oxidize specific cellular proteins as measured by 2-dimensional thiol blotting. However, the degree of protein thiol modification was not affected by a prior heat shock. Nor did the heat shock increase the intracellular concentration of glutathione or the activity of glutathione reductase. The diamide treatment caused specific, as opposed to general, protein thiol oxidation and heat shock did not prevent this.

It is hypothesized that it was the oxidation of protein thiols which led to cellular toxicity. Protein synthesis, triggered by heat shock, protected cells from the diamide toxicity without preventing protein thiol modification. These results suggest that the proteins synthesized after heat shock can provide protection against the consequences of aberrant proteins produced by thiol oxidation.

INTRODUCTION

Thermotolerance denotes a reduction in the rate of thermal inactivation triggered by an initial heat shock (1). Once triggered it takes several hours to develop and decays slowly over 24 to 72 h (2). There is some evidence that the synthesis of HSP plays a role in the production of thermotolerance; however, this point remains controversial in mammalian cells (3).

In order to understand the molecular mechanisms responsible for thermotolerance it is necessary to investigate the nature of the cellular damage responsible for thermal cytotoxicity. Based on their initial work, from which they calculated the activation energy, \( \mu_0 \), that is, 140,800 cal/mol, Westra and Dewey (4) suggested that protein denaturation may be a primary cause of cell death. This hypothesis has received support from the work of Lepock et al. (5). They measured intrinsic protein fluorescence and protein fluorophore to trans-paranaric acid energy transfer in mitochondrial and plasma membranes and demonstrated the existence of an irreversible transition in protein structure or arrangement above 40°C. Using reconstituted rabbit muscle sarcoplasmic reticulum calcium ATPase, Cheng et al. (6) have shown that inhibition of calcium uptake is due to thermal denaturation of the protein. Cholesterol or glycerol could modify the temperature of denaturation by interacting with various protein domains. These transitions may also be affected by the lipids which surround membrane proteins inasmuch as Yatvin (7) and Guffy et al. (8) have provided evidence that alteration of membrane lipids will modify thermal sensitivity. Other workers have shown that heat shock causes nuclear protein to become insoluble and bind to the nuclear matrix (9, 10). In this regard, it has been suggested that one of the functions of HSPs may be to disaggregate denatured proteins (11, 12).

One approach to the problem of determining the nature of thermotolerance and the role of HSPs is to produce very specific damage and then determine whether thermotolerant cells express resistance to it. We show here that in CHO cells the triggering of thermotolerance by a nontoxic heat shock confers protection against the toxicity caused by diamide induced protein thiol oxidation. This protection is lost when protein synthesis is inhibited.

MATERIALS AND METHODS

CHO cells growing exponentially in monolayer cultures were maintained at 37°C and at pH 7.4 in McCoy's Medium 5A supplemented with 10% fetal bovine serum, sodium bicarbonate (2.2 g/liter), penicillin G sodium (100 units/ml), and streptomycin sulfate (100 mg/ml).

Experiments were performed using T25 flasks containing 4 ml of fresh growth medium and at a cell density of approximately 4 × 10⁵ cells/flask. Both the diamide and the cycloheximide were made fresh immediately prior to use. At the end of the experimental protocol the cells were rinsed twice with Dulbecco's phosphate saline.

Cell survival was determined after trypsinizing the cells and then counting the appropriate number of cells needed to yield 100-200 colonies into T25 flasks. The error in survival was calculated according to the method of Boag (13).

If glutathione was to be measured then the cells were lysed in 1 ml of ice cold 10% PCA immediately after the saline washes. Glutathione concentrations were determined using high performance liquid chromatography (14). Cells were lysed in 3 ml of 10% PCA for protein thiol measurements. The precipitated protein was washed once in 10% PCA and twice in ice cold Dulbecco's saline. Protein thiols were quantitated using the method described by Habeeb (15) or by labeling with MPB in order to separate protein by SDS-PAGE and quantitate by thiol blotting (16).

The method described by Bayer et al. (17) and modified as follows was used for 2-dimensional thiol blots. The washed protein was solubilized in 9.5% urea, 2% Nonidet P-40, ampholines (1.6% pH 5-8; 0.4% pH 3-10), 1 mM phenylmethylsulfonyl fluoride, and 0.0367 Mg sodium (100 units/ml), and streptomycin sulfate (100 mg/ml).

Results were expressed as arbitrary units determined by comparison to standards of known concentration.

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3 The abbreviations used are: HSP, heat shock protein; MPB, 3-(N-maleimido-2-propionyl)benzoic acid; CHO, Chinese hamster ovary; GSH, glutathione; PCA, perchloric acid; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis; DTNB, dithionitrobenzene; GSSG, oxidized glutathione.
h at 20°C. The pH was measured from slices obtained from a replicate gel. Proteins were separated by molecular weight using 1.5-mm 9.5% polyacrylamide slab gels with a 4% stacking gel. The slabs were electrophoresed at 30 mA/gel, constant current at 20°C. After electrophoresis, proteins were electrophoretically transferred to nitrocellulose (20). The nitrocellulose was treated in 3% bovine serum albumin overnight, washed, incubated at room temperature for 45 min with avidin conjugated alkaline phosphatase (3 µg/ml), washed, and stained with 5-bromo-4-chloro-3-indolyl phosphate (p-toluidine salt) and nitro blue tetrazolium.

Protein synthesis was measured by labeling the cells (4 × 10⁶/flask) in 3 ml of leucine free medium containing 25 µCi/ml of [³H]leucine (specific activity, approximately 144 Ci/mol; New England Nuclear). After the labeling period, the cells were rinsed twice with 0.15 M NaCl-10 mM morpholinosulfonic acid (pH 7.4), scraped into 1 ml of the same solution, and centrifuged at 65 × g. A replicate treated similarly was used for protein determination. Cells were then dissolved in SDS-PAGE sample buffer [62 mM Tris-HCl (pH 6.8), 2% SDS, 10% glycerol, 5% 2-mercaptoethanol, and 0.0025% bromophenol blue] and heated at 95°C for 5 min. Gel lanes were loaded with approximately equal amounts of protein. Proteins were electrophoretically separated using 7.5-mm-thick 9.5% polyacrylamide gel slabs with a 4% acrylamide stacking gel in the discontinuous buffer system of Laemmli (21). Protein molecular weight was determined by comparison to molecular weight standards (myosin, M, 205,000; β-galactosidase, M, 116,000; phosphorlyase b, M, 97,400; bovine serum albumin, M, 66,000; egg albumin, M, 45,000; and carbonic anhydrase, M, 29,000). After electrophoresis, the gels were fixed (50% methanol-10% acetic acid), stained with Coomassie Brilliant Blue R250, destained, treated for fluorography using En³Hance (New England Nuclear) dried, and exposed to Kodak SB-5 X-ray film at −70°C.

Hyperthermic treatment was performed by immersion into a water bath in which the temperature was controlled ±0.05°C. Temperature was monitored with a mercury in glass thermometer traceable to National Bureau of Standards and was ±0.05°C. Irradiation was carried out using a 137Cs irradiator and a dose rate of 4.29 Gy/min.

Glutathione reductase was measured in 4 × 10⁶ cells which were lysed by freezing and thawing 3 times. The activity was measured in 1 ml of a buffer containing 3 mM morpholinosulfonic acid (pH 7.2), 12 mM MgCl₂, 54 mM KCl, 28 mM NaCl, 4.5 mM EDTA, 1.3 mM GSSG, and 0.1 mM NADPH. The decrease in absorbance at 340 nm was measured at 22°C. Experiments were repeated a minimum of two times.

RESULTS

Previous work from this laboratory (22) has shown that protein synthesis was not affected by a 15-min/43°C heat shock as assessed by incorporation of [³S]methionine into either trichloroacetic acid precipitable proteins or SDS-PAGE fluorographs quantitated by scanning densitometry. The pattern of protein synthesis observed during and up to 6 h after the 15-min heat shock was indistinguishable from controls with the exception of enhanced rates of synthesis for proteins with approximate molecular weights of 110,000, 90,000, 70,000, 60,000, and 28,000 (HSP). A 60-min/43°C heat shock, on the other hand, inhibited protein synthesis by 74%. Protein synthesis still had not returned to control levels 6 h after the heat shock. Enhanced synthesis of proteins with approximate molecular weights of 90,000 and 70,000 was only observed 4 h or more after the 60-min heat shock.

The fluorograph shown in Fig. 1 shows the pattern of protein synthesis obtained 3 h after a 15- or 60-min 43°C heat shock performed under the present experimental conditions. The resulting fluorograph obtained after [³H]leucine incorporation illustrates that 3 h after the 15-min heat shock protein synthesis was indistinguishable from control with exception of the elevated synthesis of the M, 110,000, 90,000, 70,000, and 28,000 HSPs. However, very little incorporation was observed 3 h after the 60-min heat shock (Fig. 1, Lane I). These results agree with those discussed above. Further, we have shown that thermotolerance was fully developed 3 h after the 15-min heat shock (22, 23). Both the synthesis of HSP and the development of thermotolerance were inhibited by exposure to 20 µg/ml of cycloheximide which reduced protein synthesis by 93%.

After a 43°C/15-min heat treatment followed by a 3-h incubation at 37°C, CHO cells were protected against diamide toxicity (Fig. 2). This occurred concomitantly with the development of thermotolerance (22) and the synthesis of HSP (Fig. 1) (22, 23). The cells were exposed to various concentrations of diamide 3 h after the 43°C/15-min heat shock (compare open triangles to open circles). At all concentrations tested the heat shock resulted in a statistically significant increase in survival as determined by a paired Student’s t test (P ≤ 0.05). For example, a concentration of 0.4 mM diamide reduced survival to 0.51 ± 0.04. Three h after the heat shock this concentration of diamide reduced survival to only 0.78 ± 0.05. A concentration of 0.8 mM diamide reduced survival to 0.0093 ± 0.0012. If the cells were treated with the heat shock prior to the diamide treatment then survival was 0.061 ± 0.004. A protection factor of 1.32 was obtained when the concentrations necessary to reduce survival to 10% were compared for heat shocked and non-heat shocked cells. These results, combined with those presented in Ref. 22, indicate that inhibition and recovery of protein synthesis were not necessary for protection to occur; i.e., the 43°C/15-min heat shock did not result in protein synthesis inhibition (22). Rather, this heat shock triggered HSP synthesis.

These results are in direct contrast to those obtained when the cells were exposed to a 60-min/43°C heat shock 3 h prior to the diamide treatments. Survival for this combined protocol is illustrated by the open diamonds. The 60-min heat shock killed 85 ± 10% of the cells when administered by itself. The 15-min heat shock did not produce any cell killing. When
survival obtained after the combined diamide-60-min heat shock is corrected for the heat alone toxicity then the curve shown in Fig. 2 (closed diamonds) results. This curve illustrates that in the absence of appreciable levels of protein synthesis a heat shock did not protect cells from diamide toxicity and inhibition of protein synthesis did not increase cell sensitivity to the diamide treatment.

Cycloheximide inhibition of protein synthesis inhibited heat shock induced protection against diamide toxicity. As diagramed in Fig. 3, CHO cells were subjected to one of seven protocols. Fig. 4 represents the average of 3 experiments conducted as described in Fig. 3. Protocols A, B, and C produced survival levels of 1.09 ± 0.04, 1.00 ± 0.04, and 1.00 ± 0.05, respectively, compared to untreated controls. The heat shock protected cells against either a 0.4 or 0.8 mM diamide treatment \( (P \leq 0.05) \) as determined by Student’s \( t \) test (compare Curve D to Curve F). For example, exposure to 0.4 mM diamide reduced survival to 0.59 ± 0.02. Survival after protocol F was 0.79 ± 0.02. The protection factor determined at a level of 10% survival was 1.30. The protection afforded by the heat shock was inhibited if the cells were treated with cycloheximide which inhibited protein synthesis by 93% and therefore by extension HSP synthesis (see Ref. 23, Fig. 1). This is illustrated in Fig. 4 by Curves G and E. At a survival level of 10% the protection factor was 1.10. Previous work has shown that cycloheximide did not inhibit the development of thermotolerance by 100% (23). A similar result was observed here. Heat shock protection was not completely inhibited by the addition of cycloheximide if the cells were exposed to 0.8 mM diamide. However, at a concentration of 0.4 mM survival was 0.63 ± 0.2 and 0.58 ± 0.02 \( (P \geq 0.05) \) for protocols E and G, respectively. Taken together the results shown in Figs. 2 and 4 show that protein synthesis before, during, and after heat shock is necessary for protection against diamide toxicity produced by thiol oxidation.

Diamide toxicity was enhanced if the cells were additionally exposed to cycloheximide (Fig. 4). Because diamide toxicity is directly proportional to GSH levels (data not shown) the GSH levels in cells treated with cycloheximide were measured. GSH levels fell by 26% after a 4-h incubation in 20 \( \mu \)g/ml of cycloheximide. Similar results were obtained with 100 \( \mu \)g/ml of puromycin (data not shown).

To determine whether heat shock could protect CHO cells from the oxidative damage produced by ionizing radiation, cells were incubated at 43°C for 15 min, incubated at 37°C for 3 h, and then irradiated (Fig. 5). These results demonstrate that
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Fig. 5. Dose-response curve for γ-irradiation. Cells were incubated at 43°C for either 0 (O) or 15 (A) min 3 h prior to γ-irradiation. Error bars are shown if larger than the diameter of the symbols.

Table 1 Modification of protein sulfhydryls by heat shock diamide

<table>
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<th>Protocol</th>
<th>Comment</th>
<th>nmol sulfhydryl/mg protein</th>
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<tbody>
<tr>
<td>A</td>
<td>Untreated</td>
<td>111</td>
<td>10</td>
</tr>
<tr>
<td>B</td>
<td>CHX alone</td>
<td>119</td>
<td>11</td>
</tr>
<tr>
<td>C</td>
<td>43°C/15 min</td>
<td>129</td>
<td>22</td>
</tr>
<tr>
<td>D</td>
<td>CHX alone</td>
<td>92</td>
<td>3</td>
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<td>E</td>
<td>Diamide</td>
<td>87</td>
<td>14</td>
</tr>
<tr>
<td>F</td>
<td>Diamide + CHX</td>
<td>94</td>
<td>9</td>
</tr>
<tr>
<td>G</td>
<td>43°C/15 min +</td>
<td>75</td>
<td>8</td>
</tr>
<tr>
<td>H</td>
<td>43°C/15 min +</td>
<td>60</td>
<td>5</td>
</tr>
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</table>

* Determined by the method of Habeeb (15). There were 6 flasks/point and 1 of 2 experiments.

thermotolerance and the synthesis of HSP did not protect cells from the DNA damage caused by γ-irradiation. These results agree with earlier work (24).

The 43°C/15-min heat shock did not confer protection against diamide induced inhibition of protein synthesis. Protein synthesis was inhibited by 99.2% in cells exposed to 0.8 mM diamide for 1 h at 37°C, as determined by [3H]leucine incorporated into trichloroacetic acid precipitable protein. If the cells were given a 43°C heat shock 3 h prior to the diamide treatment, inhibition was still 99.2% of control values. The heat shock by itself did not affect incorporation (data not shown). These data indicate that not all types of damage caused by diamide exposure are protected by a prior heat shock.

In order to identify which, if any, proteins were modified by the diamide treatment, proteins isolated from CHO cells after exposure to 0.8 mM diamide were analyzed by thiol blotting or by reaction with DTNB. CHO cells contained approximately 111 nmol of sulfhydryl/mg of protein (Table 1). Neither cycloheximide or heat shock or the combination affected protein sulfhydryls (Table 1, Lines A, B, and C; Fig. 6, Lanes A, B, and C). After a 1-h exposure to 0.8 mM diamide, 87 ± 14 nmol of reduced sulfhydryls/mg of protein were recovered as measured by the DTNB assay. This represents a 22% difference relative to control. However, this was not a statistically significant change (compare Lanes C0 and D in Fig. 6). Thiol blots were also obtained from cells exposed to 0.8 mM diamide for 5, 10, 15, 30, and 60 min. MPB labeling in the blots was the same as control (data not shown). The same degree of protein thiol oxidation occurred when the cells were given a 43°C heat shock 3 h prior to the diamide treatment (protocol F versus D; 75 versus 87, P > 0.05; Table 1 and Fig. 6, Lanes F and D). As shown in Fig. 4, diamide sensitivity was enhanced by exposure to cycloheximide. This was reflected in the data obtained from the sulfhydryl assay. Protocol E of Table 1 resulted in recovery of 49 ± 9 nmol of sulfhydryl/mg protein. This represents a change of 56% and is statistically significant (P < 0.05). A similar result was found in Fig. 6. These observations were not due to changes in the amount of protein recovered which stayed constant at about 0.4 mg.

The data provided in Table 1 suggest that diamide exposure resulted in a small degree of thiol oxidation. To determine whether diamide exposure produced specific oxidation rather than nonspecific or general protein oxidation, proteins were subjected to 2-dimensional thiol blotting. General or nonspecific oxidation would result if the rate of protein oxidation was the same for all proteins. Specific oxidation would be the result of different proteins having different rates of sulfhydryl oxidation. Fig. 7A represents protein thios from untreated control cells. Fig. 7B represents protein thios from cells exposed to protocol D. A comparison of the thiol blots reveals that certain specific proteins are modified by the diamide treatment. These are identified as proteins 1–5. Other proteins are also modified but they are not as prominent. The important point is that thiol oxidation or thiolation resulting from the diamide exposure appears to oxidize certain proteins to a greater extent than others.

The data obtained using DTNB as a probe for thiol modification revealed that heat shock prior to the diamide treatment did not significantly increase protein thiol modification. The same qualitative results were obtained when the corresponding thiol blots were examined. Fig. 7C represents protein thios from cells given the 43°C heat shock prior to the diamide treatment (protocol F). In summary, these results show that heat shock protected cells from the toxic effects of diamide but
not by preventing thiol modification as assessed using this technique of measurement.

The protection triggered by the heat shock treatment was not due to an increase in either intracellular GSH or an increased activity of glutathione reductase. The activity of glutathione reductase in untreated CHO cells was $2.3 \pm 0.2$ milliunits/10^6 cells. Three h after a 15-min 43°C heat shock the measured activity was $1.9 \pm 0.2$ milliunits/10^6 cells. Similarly, untreated cells contained 55 nmol of GSH/mg protein (Table 2). Immediately after treatment with 0.8 mM diamide the cells contained 18 nmol/mg (protocol D). This level of depletion (64%) by itself does not cause toxicity in CHO cells (25). Heat shock alone or administered 3 h prior to the diamide treatment did not affect GSH levels.

DISCUSSION

The object of this investigation was to produce a specific type of cellular damage and then determine whether resistance would be observed as cells developed thermotolerance and synthesized HSP. This was accomplished by incubating CHO cells at 43°C for 15 min and then exposing the cells to diamide 3 h later. Three h after this heat shock thermotolerance has been shown to be fully developed and dependent on protein synthesis (22, 23). This heat shock also triggered the development of diamide resistance. Resistance occurred to only a specific type of damage. Resistance was not observed to diamide inhibition of protein synthesis or DNA damage produced by γ-irradiation.

The data presented in this current investigation have shown that diamide produces thiol oxidation of specific proteins as measured by 2-dimensional thiol blot analysis. These observations agree with those obtained by Grimm et al. (26) who looked at protein mixed disulfides in rat cardiac cells. They found that addition of 0.5 mM diamide to the cultured rat cells produced S-thiolation of at least 21 proteins. However, not all proteins were S-thiolated equally and some proteins were not modified at all. Diamide reacts with acidic, small molecular weight thiols such as glutathione. In general, protein thiols would not be expected to be directly oxidized because they are generally less acidic and sterically less accessible. This does not preclude specific proteins from reacting directly with diamide. Most protein oxidation, however, will be due to the formation of mixed disulfides. Kosower et al. (27) have carefully calculated the rate constants for reaction of diamide with compounds other than glutathione (e.g., NADH, NADPH, or lipoic acid). They found that the fastest reaction occurred with glutathione; it was approximately 87 times faster than that which occurred with NADH or NADPH. Only after glutathione depletion has occurred would diamide be expected to react with other cellular compounds. The data shown in Table 2 of the present study indicate that the diamide exposure depleted GSH levels by only 64%. GSH levels were also assayed 5, 10, 15, or 30 min into the diamide exposure interval and depletion never exceeded 64% (data not shown). Thus, diamide would be expected to react only with GSH to form GSSG and possibly certain specific protein thiols. Formation of GSSG could result in increased protein thiol oxidation via glutathione protein mixed disulfides. In this regard, Kosower and Kosower (28) have shown that in human RBC formation of intra- and interchain disulfide bonds in proteins is directly related to the rate of oxidation of GSH to GSSG. One further consideration is that diamide exposure will result indirectly in the reversible oxidation of NADPH via

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**Table 2 GSH levels in CHO cells**

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<th>Protocol</th>
<th>Comments</th>
<th>nmol GSH/mg protein</th>
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<tr>
<td>D</td>
<td>Untreated</td>
<td>55</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Diamide alone</td>
<td>18</td>
<td>6</td>
</tr>
<tr>
<td>B</td>
<td>43°C/15 min</td>
<td>43</td>
<td>7</td>
</tr>
<tr>
<td>F</td>
<td>43°C/15 min + diamide</td>
<td>20</td>
<td>1</td>
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glutathione reductase, potentially altering the pyridine nucleotide redox position. However, the major consequence of diamide treatment must be considered to be an increase in the formation of GSSG.

The mechanism responsible for the diamide toxicity is not known. Toxicity is directly related to redox potential in CHO cells. For example, when cells were exposed to 0.1 mM diamide for 1 h at 37°C in Hank's balanced salt solution in the absence of glucose, survival was 0.02 ± 0.005. Addition of 2 mM glutamine or 10 mM glucose, as a source for NADPH regeneration, increased survival to 0.3 ± 0.02 and 1.00 ± 0.03, respectively (data not shown). It is reasonable to speculate that toxicity is a consequence of thiol oxidation but other possibilities cannot be excluded. The data shown in Fig. 7 and Table 1 show that exposure to diamide produced specific thiol oxidation. Exposure to 0.8 mM diamide killed more than 90% of the cells yet oxidized only 22% of the proteins. The most prominent oxidized proteins are identified in Fig. 7. It would be expected that oxidation of only some of these proteins results in disruption of the tertiary structure. It is the distortion of protein structure that would allow these proteins to be candidates for the repair by HSP synthesis in accordance with the model of HSP function (12). Thus it may be expected that thermotolerance would protect only a small number of proteins.

Previous work has shown that thiol oxidation upon exposure to diamide triggers both the development of thermotolerance and the synthesis of HSP in CHO cells (29, 30). Diamide is not the only oxidant to do this. Exposure to H2O2 also triggers HSP and tolerance in Salmonella typhimurium and CHO cells (31, 32).

When CHO cells were triggered to develop thermotolerance and synthesize HSP by administration of a nontoxic heat shock, they also developed resistance to diamide. The development of diamide resistance was inhibited if the cells were treated with cycloheximide or a heat shock which inhibited protein synthesis. Cycloheximide has been shown to inhibit the development of thermotolerance in CHO cells. Further, resistance could not be attributed to an increase in the activity of glutathione reductase or to an increase in the glutathione concentration. Thus, cells exhibited resistance to diamide toxicity when they developed thermotolerance and synthesized HSP, and resistance failed to develop when tolerance and protein synthesis were inhibited. These results suggest that it is the development of thermotolerance and, by extension, the synthesis of HSP which confers resistance to the toxicity caused by oxidation of specific protein thiols.

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