

# Enhanced synthesis of heat shock proteins and augmented thermotolerance after induction of differentiation in HL-60 human leukemia cells

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The effects of the induction of differentiation were investigated on the expression of heat shock proteins (hsps) and thermotolerance. The synthesis of the major hsps in response to heat stress was markedly enhanced in HL-60 human leukemia cells after differentiation. An increased amount of mRNA transcripts for hsp70 was also noted. In addition, induction of differentiation resulted in acquisition of greater resistance to heat, which may be advantageous since cells in the peripheral blood must survive many stresses.

Heat shock protein; Differentiation; Thermotolerance; HL-60 cell

## INTRODUCTION

Heat shock proteins (hsps) are a specific set of proteins induced by hyperthermia and other stresses. The most prominent hsps in mammalian cells have molecular masses of approximately 90000 (hsp90) and 70000 (hsp70) [1]. Besides environmental insults such as heat, the expression of specific hsps is also regulated by cellular differentiation or development [2–4].

It has long been proposed that hsps are responsible for thermotolerance [5,6]. Although the molecular mechanism(s) involved in thermotolerance are unclear, a good correlation has been established between increased synthesis of hsps and the development of thermotolerance [7].

To determine whether or not differentiation affects the resistance of cells to heat, we studied the effects of differentiation on the expression of hsps and thermotolerance in HL-60 cells, a well-characterized human leukemia cell line which can be differentiated into mature granulocytic cells by treatment with dimethyl sulfoxide (DMSO) [8].

## 2. MATERIALS AND METHODS

### 2.1. Cell culture and induction of differentiation

HL-60 cells were provided by the Japanese Cancer Research Resources Bank (Tokyo, Japan). For differentiation experiments, cells were suspended at  $2 \times 10^5$  cells/ml in RPMI 1640 medium sup-

plemented with 10% fetal calf serum (FCS) and treated with 1.3% (v/v) DMSO (Wako Pure Chemical Industries Co., Osaka, Japan) for the indicated lengths of time.

### 2.2. Heat shock and cell labelling

DMSO-treated or untreated cells were washed with phosphate-buffered saline and resuspended at  $5 \times 10^6$  cells/ml in methionine-free Eagle's minimal essential medium containing 10% dialyzed FCS. Cells in microcentrifuge tubes were submerged in a water bath at 42°C for 1 h, then labeled for 1 h at 42°C by the addition of [<sup>35</sup>S]methionine (1094 Ci/mmol, American Radiolabeled Chemicals, St. Louis, USA) at 20 µCi/ml. After labeling, the cells were washed and total cellular extracts were lysed for electrophoresis. For assessing the level of protein synthesis, cells with or without DMSO treatment were washed, resuspended at  $6 \times 10^5$  cells/ml in RPMI 1640 with 10% FCS, and immersed in a water bath at 37°C or 43°C for 1 h. Following recovery at 37°C for varying periods as indicated, the cells were washed and labeled with [<sup>35</sup>S]methionine at 37°C for 30 min, as described above. After labeling, the cells were washed and protein was precipitated by adding trichloroacetic acid (TCA) to a final concentration of 10%. The precipitates were washed with 10% TCA and solubilized in 1 N NaOH. The radioactive protein content of the solutions was then determined by scintillation counting. This was divided by the total protein content determined by the method of Lowry et al. [9] to determine the relative incorporation of the label into protein.

### 2.3. Immunoprecipitation

[<sup>35</sup>S]Methionine-labeled proteins were immunoprecipitated by the addition of rabbit antiserum against mouse hsp90, as described previously [10]. The anti-hsp90 serum was kindly provided by Dr S. Koyasu (The Tokyo Metropolitan Institute of Medical Science) [11].

### 2.4. Gel electrophoresis

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) followed by fluorography was performed as described previously [10].

### 2.5. Northern blot analysis

Total cellular RNA was isolated by a guanidinium isothiocyanate procedure and cesium chloride centrifugation [12]. Denaturation,

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fractionation, and transfer of total RNA were performed according to the method of Thomas [13]. c-myc,  $\beta$ -actin, and pHS709 (a gift of Drs E Hickey and L. Weber, University of South Florida, which contains a gene coding for human hsp70 [14]) were used as probes for Northern blotting. After hybridization was carried out, the membrane was processed for autoradiography.

### 3. RESULTS AND DISCUSSION

#### 3.1. Effects of differentiation on the expression of hsp

Induction of differentiation by DMSO enhanced the synthesis of hsp90 and hsp70 in response to heat shock (Fig. 1). In addition, the suppression of the synthesis of constitutive proteins, such as actin, was less severe in the differentiated HL-60 cells. Northern blot analysis showed (although less markedly than the result obtained by protein analysis) the increased induction of hsp70 mRNA in differentiated cells (Fig. 2) in which c-myc expression was markedly decreased [15]. In mouse embryonal carcinoma cells, it has also been noted that cells produced more hsps after the induction of differentiation than did undifferentiated cells [4]. Therefore, enhancement of the synthesis of hsps during differentiation might be a common phenomenon irrespective of cell type.

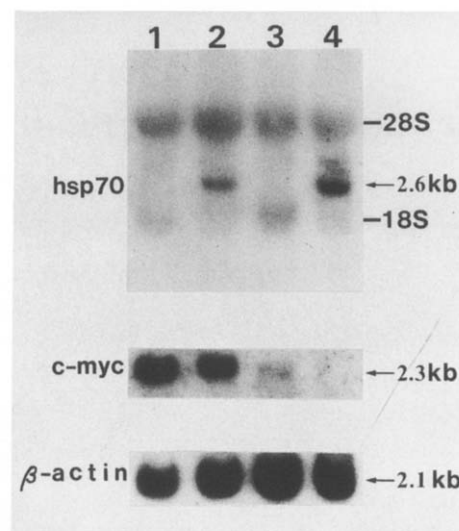


Fig. 2. Northern blot analysis of hsp70 mRNA from HL-60 cells undergoing various treatments. Equal amounts of total RNA (10  $\mu$ g) from cells incubated for 2 h at 37°C or 42°C were applied to each lane and probed with a cDNA for human hsp70. (Lane 1) untreated cells at 37°C; (lane 2) untreated cells at 42°C; (lane 3) DMSO-treated cells at 37°C; (lane 4) DMSO-treated cells at 42°C. To confirm differentiation and the equality of the amounts of RNA loaded, the blots were rehybridized with c-myc and  $\beta$ -actin, respectively.

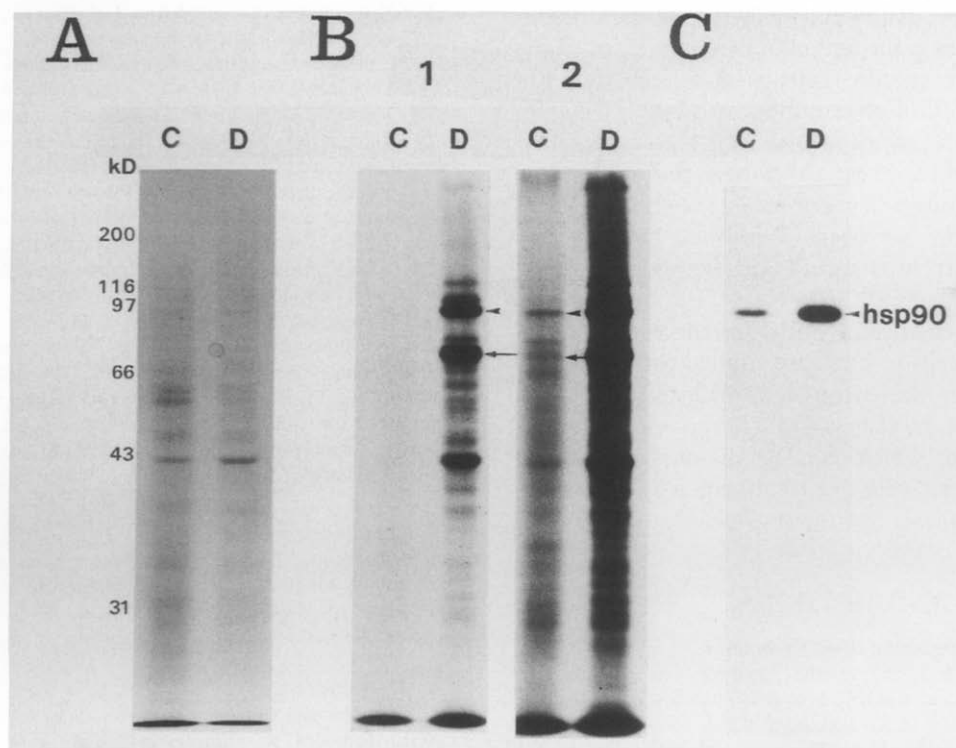


Fig. 1. Electrophoretic analysis of [ $^{35}$ S]methionine-labeled proteins synthesized during heat shock from control (C) and DMSO-treated (D) HL-60 cells. (A) Coomassie blue staining of 10.5% SDS-PAGE. Equal numbers of cells ( $2 \times 10^5$ ) were applied to each lane. (B) Fluorograms of the gel shown in (A). (1) 24-h exposure; (2) 5 days' exposure. Arrowheads and arrows indicate hsp90 and hsp70, respectively. (C) Immunoprecipitation with antiserum against hsp90. Cell lysates obtained from equal cell numbers ( $4 \times 10^5$ ) were immunoprecipitated. The precipitates were subjected to SDS-PAGE and visualized by fluorography.

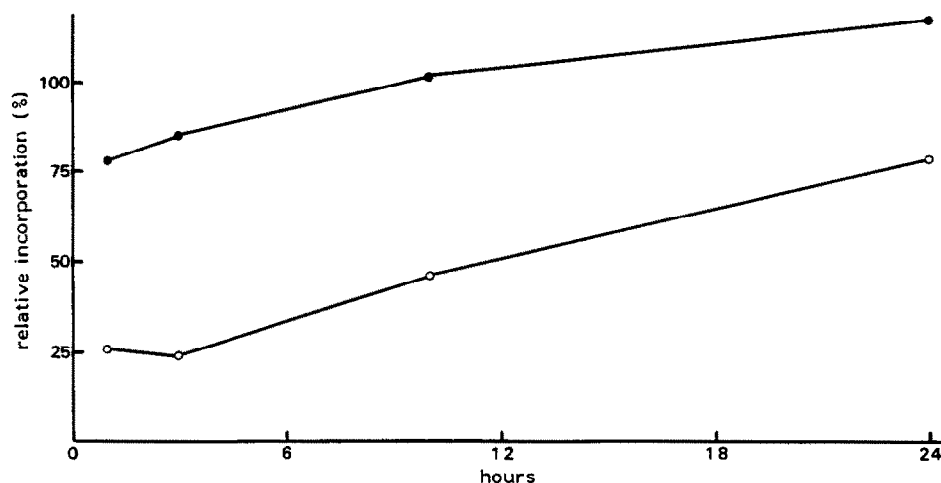


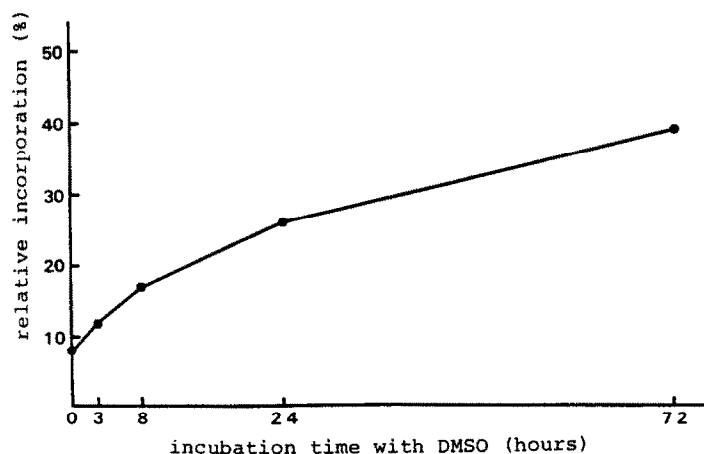
Fig. 3. Recovery of protein synthesis following heat shock in untreated (○—○) and DMSO-treated (●—●) HL-60 cells. Incorporation of the label was determined for duplicate samples and expressed as an average and relative to that seen in cells given no heat treatment.

### 3.2. Changes in the recovery of protein synthesis following heat shock after induction of differentiation

To determine whether the increased synthesis of hsp's in differentiated cells results in the acquisition of greater thermotolerance, we compared the recovery of protein synthesis after heat shock in DMSO-treated and untreated HL-60 cells. The overall protein synthesis of untreated cells was profoundly suppressed immediately after heat shock and did not recover to the control level even after 24 h (Fig. 3). On the other hand, the suppres-

sion of protein synthesis in DMSO-treated cells was much less severe as seen in Fig. 1, and full recovery of protein synthesis was observed as early as 12 h following heat shock. Previous studies have shown that the recovery kinetics of protein synthesis at 37°C after heat shock correlates well with the thermotolerance of cells [2,16,17]. In this light, DMSO-treated HL-60 cells appeared to be more resistant to heat than untreated cells. It was also likely that the acquired thermotolerance in DMSO-treated cells resulted from increased synthesis of hsp's during the process of differentiation. However,

**A**



**B**

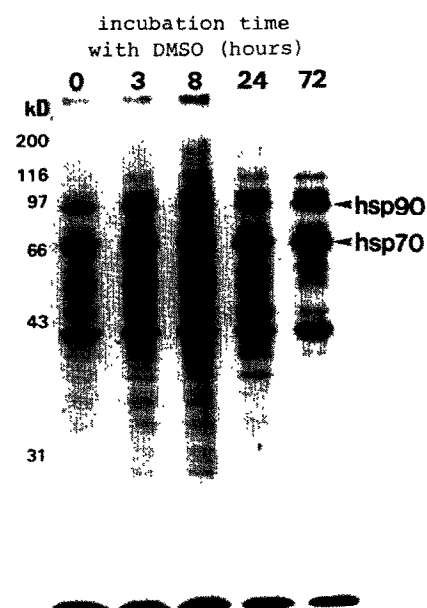


Fig. 4. Effects of the length of incubation with DMSO on protein synthesis following heat shock in HL-60 cells. (A) Kinetics of overall protein synthesis. Proteins synthesized in cells incubated for various times with DMSO were labeled with [<sup>35</sup>S]methionine at 37°C immediately after heat shock. Incorporation of the label was expressed relative to that seen in cells given no heat treatment. (B) Time-course comparison of hsp's synthesized in cells incubated for various times with DMSO. Equal amounts of radioactivity were loaded in each lane.

it was also possible that thermotolerance was related to changes in cell membrane components, since DMSO is an organic solvent that is known to affect thermotolerance independent of cellular differentiation [18]. To distinguish between the effects of differentiation and the direct effects of DMSO, we compared the responses of cells incubated for various times with DMSO. Immediate suppression of overall protein synthesis after heat shock was gradually reduced in intensity as the duration of incubation with DMSO increased (Fig. 4 A). Synthesis of major hsps, especially of hsp70, was also gradually enhanced in proportion to the length of incubation with DMSO (Fig. 4B). These results suggested that the augmented thermotolerance and the enhanced synthesis of hsps could be attributed to the effects of differentiation, rather than to a direct effect of DMSO which would be expected to be observed relatively rapidly. The significance of the acquisition of thermotolerance during differentiation of white blood cells remains to be determined. It may be advantageous because cells in the peripheral blood have to survive exposure to many stresses, including temperature changes.

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