

The human 32-kDa stress protein induced by exposure to arsenite and cadmium ions is heme oxygenase

Shigeru Taketani, Hirao Kohno, Takeo Yoshinaga* and Rikio Tokunaga

*Department of Hygiene, Kansai Medical University, Moriguchi, Osaka 570 and *Department of Public Health, Faculty of Medicine, Kyoto University, Sakyo-ku, Kyoto 606, Japan*

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Exposure of HeLa and HL60 cells to sodium arsenite or cadmium chloride led to marked increases in cellular heme oxygenase activity. SDS-polyacrylamide gel electrophoresis of [³⁵S]methionine-labeled cellular proteins indicated that these treatments also resulted in the induction of a 32-kDa protein. Immunoblot analysis further showed that the 32-kDa protein reacted with anti-bovine heme oxygenase antibodies. Treatment of the cells with cobaltic chloride or heat induced neither the 32-kDa protein nor heme oxygenase activity. It is concluded that the 32-kDa stress protein induced by arsenite and cadmium ions in these human cells is heme oxygenase.

Stress protein, 32 kDa; Heme oxygenase; Arsenite; Cadmium ion; (Human cell)

1. INTRODUCTION

Exposure of pro- and eukaryotic cells to heat shock or other stress such as arsenite and amino acid analogues results in the synthesis of several heat-shock (stress) proteins [1–4]. Stress proteins having molecular masses of 70–90 kDa appear to be induced under most stress conditions, but the other stress proteins are induced only under restricted stress conditions. For instance, exposure of human and mouse cells to arsenite and cadmium ions, but not heat shock, has been reported to lead to the induction of 32- and 34-kDa proteins, respectively [5,6]. The functions of these proteins are, however, not known.

Heme oxygenase catalyzes the oxidative degradation of protoheme to biliverdin [7] and has a molecular mass of 32 kDa [7,8]. In the rat, this enzyme is induced by various metal ions [9,10] and

bromobenzene [11]. Shibahara et al. [12] have recently reported the induction of the oxygenase in rat glioma cells by heat-shock treatment. We have also shown that exposure of rat hepatoma cells to heavy metal ions as well as to heat-shock treatment causes marked induction of heme oxygenase [13]. Unlike the case of the rat enzyme, heat shock has been reported to cause no induction of human heme oxygenase [14].

In this study, we examined the effects of arsenite, cadmium ions, cobaltic ions and heat shock on the synthesis of heme oxygenase in HeLa and HL60 cells, two human cell lines. The results obtained provide evidence that the 32-kDa protein induced by arsenite and cadmium ions is heme oxygenase.

2. MATERIALS AND METHODS

2.1. Materials

L-[³⁵S]Methionine (800 Ci/mmol) was purchased from New England Nuclear. RPMI 1640 medium, MEM, and fetal calf serum were obtained from Gibco. All other chemicals used were of reagent grade. Anti-bovine heme oxygenase antibodies were prepared as described [7].

Correspondence address: S. Taketani, Department of Hygiene, Kansai Medical University, Moriguchi, Osaka 570, Japan

Abbreviation: MEM, Eagle's minimum essential medium

2.2. Cell culture and exposure to stress

Human leukemic HL60 cells were grown in suspension culture as described [15] to a cell density of 2×10^5 /ml, and the cells harvested were exposed to arsenite or metal salts in RPMI 1640 medium containing 10% fetal calf serum at 37°C for 8 h. HeLa cells were grown at 37°C in MEM supplemented with 10% fetal calf serum. The confluent cultures were exposed to arsenite or metal salts in fresh MEM containing 10% fetal calf serum at 37°C for 8 h. The cell cultures were heat-shocked by adding prewarmed fresh medium (43°C) and maintained in a CO₂ incubator adjusted to 43°C for 2 h.

2.3. Metabolic labeling of cells and immunoblotting

The cells (5×10^6) were incubated at 37°C for 8 h in methionine-free RPMI 1640 medium containing dialyzed fetal calf serum [16], [³⁵S]methionine (50 μCi), and, when necessary, arsenite or metal salts. The cells were washed twice with phosphate-buffered saline and lysed with Laemmli's sample buffer [17]. SDS-polyacrylamide gel electrophoresis was performed by the method of Laemmli [17] using 10% gel. The gels were fixed, dried and subjected to fluorography using Kodak X-ray film. Immunoblotting was carried out as described [13].

2.4. Other methods

Heme oxygenase activity was determined as described [13]. Protein concentration was estimated by the method of Lowry et al. [18].

3. RESULTS

As shown in table 1, treatment of HeLa cells with arsenite and cadmium ions caused 8- and 6-fold increases in cellular heme oxygenase activity, respectively. In HL60 cells, in which the basic heme oxygenase activity was very low, the oxygenase activity was markedly induced by cadmium chloride. Arsenite also caused an increase in the activity, though to a lesser extent. In both types of cells, no increase in the activity was observed upon exposure to cobaltic ions or heat-shock treatment.

HeLa cells were incubated with 50 μM sodium arsenite or 10 μM cadmium chloride in the presence of [³⁵S]methionine, and radioactive cellular proteins were analyzed by SDS-polyacrylamide gel electrophoresis, followed by fluorography (fig.1A). As can be seen, treatment of the cells with arsenite and cadmium ions both caused marked increases of the 32-kDa protein (lanes 2 and 3). A similar increase of the 32-kDa protein was also observed in HL60 cells upon exposure to arsenite and cadmium ions (fig.1B). However, no increase of the 32-kDa protein was detected in either cell type when exposed to 50 μM

Table 1

Heme oxygenase activity in human cell lines by arsenite, metal ions and heat-shock treatment

Treatment	Heme oxygenase activity ^a (nmol/mg of protein per h)	
	HeLa cells	HL60 cells
None	0.31	<0.1
Sodium arsenite (50 μM)	2.44	0.45
Cadmium chloride (10 μM)	1.96	2.90
Cobaltic chloride (50 μM)	0.33	<0.1
43°C, 2 h	0.25	<0.1

^a The values were the average of duplicate experiments

Experimental conditions are described in section 2

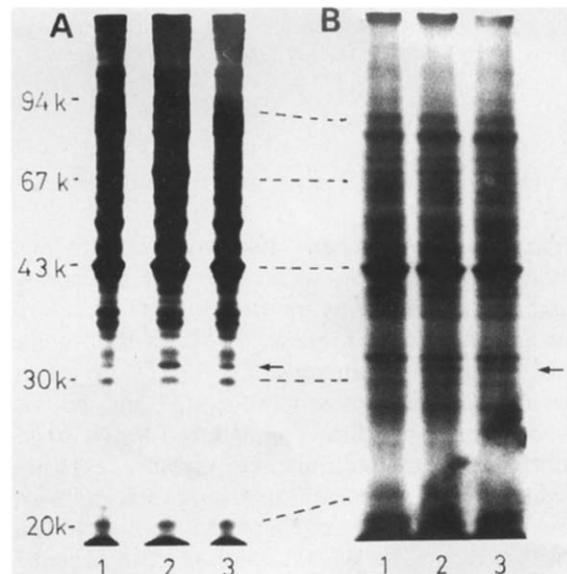


Fig.1. Induction of the 32-kDa protein in HeLa and HL60 cells by exposure to sodium arsenite and cadmium chloride. The cells were incubated with or without the indicated inducers in the presence of [³⁵S]methionine. The cells were then lysed and labeled proteins were analyzed by SDS-polyacrylamide gel electrophoresis and subsequent fluorography. (A) HeLa cells; (B) HL60 cells. Lanes 1, 2 and 3 indicate the results obtained with untreated cells, cells treated with 50 μM sodium arsenite, and cells treated with 10 μM cadmium chloride, respectively. The arrows show the 32-kDa protein band. Marker proteins used were phosphorylase *b* (94 kDa), bovine serum albumin (67 kDa), ovalbumin (43 kDa), carbonic anhydrase (30 kDa) and trypsin inhibitor (20 kDa).

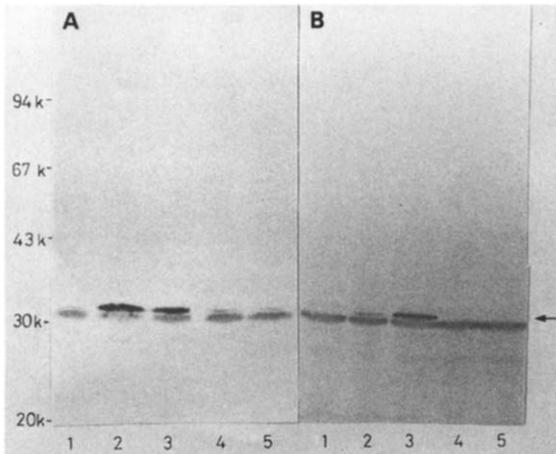


Fig.2. Immunoblot analysis of the induced 32-kDa protein in HeLa and HL60 cells with anti-bovine heme oxygenase antibodies. (A) HeLa cells; (B) HL60 cells. Lanes: 1, untreated cells; 2, cells treated with 50 μ M sodium arsenite; 3, cells treated with 10 μ M cadmium chloride; 4, cells treated with 50 μ M cobaltic chloride; 5, cells heat-shocked at 43°C for 2 h. Immunoblotting was performed as described [13]. The arrow indicates the heme oxygenase band.

cobaltic chloride or heat-shock treatment (not shown).

Fig.2 shows the immunoblot analysis results of heme oxygenase in the cells exposed to arsenite, metal ions and heat shock. After treatment of HeLa cells with arsenite or cadmium ions, anti-bovine heme oxygenase antibodies clearly recognized the 32-kDa protein (fig.2A, lanes 2 and 3). However, no immunoreactive protein was detected in HeLa cells exposed to cobaltic ions or heat-shock treatment (lanes 4 and 5). The additional 31-kDa band was observed in all lanes, and crossreactivity of this protein could not be totally prevented. Similar results were obtained with HL60 cells, although cadmium ions were more effective than arsenite in inducing the immunoreactive 32-kDa protein (fig.2B).

4. DISCUSSION

The results described above leave little doubt that the 32-kDa protein induced in HeLa and HL60 cells upon exposure to arsenite and cadmium ions is heme oxygenase. This conclusion is further supported by the findings reported by Caltabiano et al. [5] that the 32-kDa stress protein induced in

human cells by arsenite and cadmium ions is a membrane protein, into which no detectable incorporation of [³⁵S]cysteine can be observed. As is well known, heme oxygenase is a microsomal membrane protein and the primary structures of the rat and human enzymes, deduced from cDNA nucleotide sequences, do not contain any cysteine residues [14,19]. Moreover, Keyse and Tyrrel [6] have reported that the 32-kDa stress protein is induced in human fibroblasts by arsenite, hydrogen peroxide and ultraviolet radiation, but not by heat-shock treatment. Caltabiano et al. [5] have also shown that the 32- and 34-kDa stress proteins in human and mouse melanoma cells, respectively, are induced by arsenite and heavy metal ions such as copper and cadmium ions and this induction is not effected by heat shock. These results are consistent with those obtained in the present study. Taken together, it can be definitely concluded that the 32-kDa stress protein is heme oxygenase.

We have previously reported that both heavy metal ions and heat shock can induce heme oxygenase in rat hepatoma cells [13]. The present study as well as the report by Yoshida et al. [14] indicate that heat-shock treatment is not capable of inducing the enzyme in human cells. Furthermore, the results obtained in this study indicate that cobaltic salt, which is a potent inducer of heme oxygenase in the rat [9,10,13], is ineffective in inducing the enzyme in human cells. The molecular mechanism underlying such species-specific induction and the possible functional role of heme oxygenase under stress conditions still remain to be elucidated.

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