

# Effect of Prostaglandin A<sub>1</sub>, Arsenite and Aspirin on Stress Proteins Response in Mosquito Cells

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The stress response of eukaryotic cells is characterized by changes in the metabolism of responding cells, most notably by increased synthesis of a group of proteins known as heat shock (HSP) proteins. In this paper the effect of prostaglandin A<sub>1</sub> (PGA<sub>1</sub>), arsenite and aspirin in *Aedes albopictus* cells was investigated. In cells treated with PGA<sub>1</sub> (10 µg/ml) we observed the induction of several polypeptides with molecular masses of 87, 80, 70, 57, 29 and 23 kDa. Immunoblot analysis revealed that arsenite induces a marked synthesis of HSP70, and aspirin administered during the hyperthermic treatment caused a small increase of HSP70 synthesized.

## Introduction

A wide variety of organisms and cell types, when submitted to temperatures higher than their normal growing temperature, induce the synthesis of a new set of proteins, while formation of most other cellular proteins ceases. These proteins are well characterized and are referred to as heat shock proteins. As the term heat shock has restrictive connotations, actually this phenomenon has become more generally known as the stress response (Schlesinger *et al.*, 1982; Csermely, 1998).

The induction or enhanced synthesis of heat shock proteins (or stress proteins) observed in response to environmental stresses, including heat, chemical, or mechanical treatments, has been reported in a wide variety of cells ranging from yeast and *Drosophila* to mammalian cells. These proteins can be divided into five families with Mr of 15–30 kDa (low Mr HSP), 60 kDa (HSP 60), 70 kDa (HSP 70), 90 kDa (HSP 90) and 100–110 kDa (HSP 110) respectively. In eukaryotic cells, HSPs are generally present as multigenic families, consisting of closely related protein isoforms, with members being expressed in unstressed cells (constitutive HSPs) as well as by a following heat treatment (inducible HSPs) (Linguist and Craig, 1988; Schlesinger, 1990).

Induction of HSPs requires the activation, translocation to the nucleus and phosphorylation of

a regulatory protein, the heat shock transcription factor (HSF), which binds to multiple arrays of heat shock elements (HSEs) located in the promoters of heat shock genes (Morimoto *et al.*, 1992).

The induction of stress proteins in mosquito cells is poorly investigated. However, the induction of heat-shock proteins during the growth of *Aedes albopictus* cells has been previously reported. Heat-shock treatment of these cells produces a drastic alteration in the pattern of protein synthesis which is a function of cellular growth (Carvalho and Rebello, 1987).

The effect of prostaglandin, arsenite and aspirin in mammalian cells has been intensively studied. In this paper we investigate the effect of these drugs in the induction of HSP70 in mosquito (*Aedes albopictus*) cells.

## Materials and Methods

### Cell cultures

*Aedes albopictus*, clone C6/36 were used in these studies (Igarashi, 1978). This cell line was a gift from Arbovirus Research Unit, Yale University, USA. These cells were grown in glass bottles with a 60 cm<sup>2</sup> opening at 28 °C during 72 h. The growth medium consisted of Leibovitz's (L-15) supplemented with 0.2 mM non-essential amino



acids, 0.3% tryptose phosphate broth, 0.02% L-glutamine, 10% fetal calf serum, penicillin (500 U/ml), streptomycin (100 µg/ml) and amphotericin B (fungizone 2.5 µg/ml). For subcultivations, confluent monolayers containing  $1.5 \times 10^7$  cells/bottle were gently washed with Dulbecco's phosphate-buffered saline (PBS) and after a short trypsinization the cells were suspended in the culture medium. The monolayers, grown in scintillation vials with a 5 cm<sup>2</sup> area were seeded with  $3.5 \times 10^5$  cells and the culture incubated at 28 °C. Prostaglandin A<sub>1</sub>, sodium arsenite and aspirin (acetylsalicylic acid) were purchased from Sigma Chemical Co, St. Louis, MO.

#### *Heat shock treatment and labelling of cultures with [<sup>35</sup>S]methionine*

*Aedes albopictus* cells growing (at 28 °C) in scintillation vials were preincubated during 30 min at 37 °C in Eagle's minimal essential medium in the absence of serum and methionine. After this period, the medium was supplemented with [<sup>35</sup>S]methionine (0.74 MBq/ml) and the incubation was continued. One hour later, the medium was removed, the monolayers were washed with PBS and the cells treated with loading buffer. (63 mM Tris-HCl, hydroxymethylaminomethane, pH 6.8; 2% sodium dodecyl sulfate; 10% glycerol; 5% 2-mercaptoethanol and 0.001% bromophenol blue).

#### *Analysis of [<sup>35</sup>S]methionine labelled proteins by polyacrylamide gel electrophoresis*

Cell proteins labelled with [<sup>35</sup>S]methionine as described above were directly resuspended in 70 µl of loading buffer. Samples were then heated for 5 min at 95 °C and subjected to electrophoresis on one-dimensional 12.5% polyacrylamide gels using the SDS buffer system of Laemmli (1970) at room temperature. The dried gels were exposed to Kodak X-Omat (YAR-S) film. The molecular mass of proteins was determined by co-electrophoresis of standard proteins (Pharmacia).

#### *Immunoblot analysis*

For immunoblot analysis, an equal amount of protein from each sample was separated by SDS-PAGE as described above, and blotted onto nitrocellulose using the technique described by Towbin

*et al.* (1979). After transfer the filters were incubated with an anti HSP70 monoclonal antibody (Sigma Chemical Co.). The bound antibody was detected using peroxidase-linked antimouse antibody (Amersham). Molecular weights were calculated using Mr markers from Pharmacia.

### **Results and Discussion**

We examined the effects of PGA<sub>1</sub> on protein synthesis in *Aedes albopictus* cells. Cells were treated with 10 µg/ml of this drug, labelled with [<sup>35</sup>S]methionine and the proteins analyzed by polyacrylamide gel electrophoresis. In Fig. 1 we compared the proteins induced by PGA<sub>1</sub> (lane C) with those induced by thermal treatment (lane B). In cells treated with PGA<sub>1</sub> we observed an increase in the synthesis of HSPs 87, 80 and 70 kDa compared with control cells (lane A). In addition, PGA<sub>1</sub> induced the synthesis of proteins (57 and 23 kDa) which were not observed in heat-shocked cells (lane B) and failed to induce the low molecular weight HSPs (29 and 27 kDa). Proteins p57 and p23 represent stress proteins whose expression is primarily regulated by PGA<sub>1</sub>, but not by hyperthermia. A low molecular weight stress protein was also described by Koizumi *et al.* (1991) who found that PGD<sub>2</sub> and PGJ<sub>2</sub> stimulated porcine aortal endothelial cells to synthesize a 31 kDa protein.

A correlation between heat shock and arachidonic acid metabolism was demonstrated by Calderwood *et al.* (1989) in mammalian cells. Heat shock stimulated the liberation of arachidonic acid at temperatures above 39° C and led to formation of prostaglandin E<sub>2</sub> and F<sub>2</sub>, and leukotriene B<sub>4</sub>.

While eicosanoids are very well known in mammalian systems, there is increasing recognition of the significance of these compounds in insects and other invertebrates. Eicosanoids exert physiological actions in reproduction of some insects, hatching in barnacles, egg production in snails, spawning in bivalves, oocyte maturation in sea stars and prevention of polyspermic fertilizations in sea urchin eggs. Eicosanoids are also involved in salt and water transport physiology in insects and bivalves. (reviewed in Stanley-Samuelson, 1994; Stanley-Samuelson and Pedibhotla, 1996).

Next we examined the effect of sodium arsenite on the induction of stress proteins (Fig. 2). A direct comparison was made between the proteins

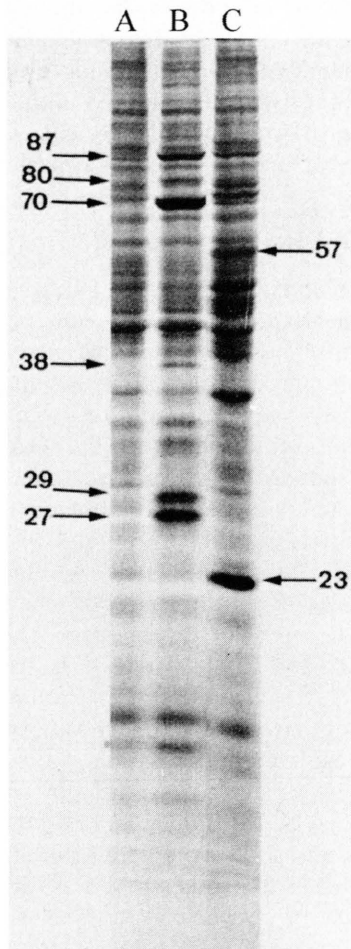


Fig. 1. Effect of  $\text{PGA}_1$  on protein synthesis of *Aedes albopictus* cells. Monolayers were labelled with [ $^{35}\text{S}$ ]methionine (0.74 MBq/ml) and the proteins subjected to SDS-PAGE as described. Lane A represents untreated control cells maintained at 28 °C; lane B, heat shocked cells (37 °C) and lane C, cells treated with  $\text{PGA}_1$  (10 µg/ml) for 12 h.

induced by heat shock,  $\text{PGA}_1$  and arsenite. After this treatment, cells were lysed and equal amounts of protein from each sample separated by SDS-PAGE and processed for immunoblot analysis, using monoclonal antibody (anti-HSP70). Immunoblot analysis revealed the presence of a unique band. Arsenite (lane D and E) induced the synthesis of HSP70 which co-migrated with the protein induced by heat shock (lane B) or  $\text{PGA}_1$  (lane C). Actinomycin D treatment completely suppressed the induction of HSP70 mediated by heat,  $\text{PGA}_1$  and arsenite (data not shown).

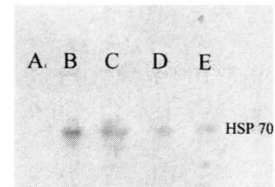


Fig. 2. Effect of  $\text{PGA}_1$  and sodium arsenite in the induction of HSP70 protein in *Aedes albopictus* cells. Lane A represents untreated cells, maintained at 28 °C, lane B heat shocked cells (37 °C) and lane C cells treated with  $\text{PGA}_1$  (10 µg/ml) for 12 h. Lanes D and E represent cells treated with sodium arsenite (2.5 µM) during 1 and 3 hours, respectively. HSP70 synthesis in samples was identified by immunoblot analysis as described in Material and Methods.

The induction of proteins by arsenite has been studied in several types of cells. (Johnston *et al.*, 1980; Salzman and Bowman, 1992; Deaton *et al.*, 1990; Levinson *et al.*, 1980; Taketani *et al.*, 1989) however, the molecular mechanism involved is not completely clear. Salzman and Bowman (1992) found that in human (W138 embryonic lung) cells, arsenite increased the synthesis of arachidonic acid metabolites such as prostaglandins  $\text{E}_2$  and prostacyclin. Arsenite reacts with sulfhydryl moieties of proteins (Fluharty and Sanadi, 1960) and may interact at the cell surface with membrane receptors of enzymes involved in the regulation of prostaglandin synthesis.

As reported by other investigators the anti-inflammatory agent sodium salicylate induces heat shock responsive chromosomal puffs in *Drosophila* salivary glands and stimulates heat shock transcription factor (HSF) DNA binding activity in cultured *Drosophila* cells. (Ritossa, 1963; Zimmarino and Wo, 1987). Amici *et al.* (1995) found that aspirin induce HSF-DNA binding activity and enhance thermotolerance in human erythroleukemic (K562) cells.

Previous results from our laboratory demonstrated that aspirin did not induce stress proteins in *Aedes albopictus* cells (unpubl. results). With the objective to verify whether aspirin (400 µM) potentiates the heat shock response, we added this compound to the cells maintained at 28 °C or at 37 °C for 1h. After this treatment samples were processed for immunoblot analysis. HSP70 was quantified by densitometric analysis. As shown by Fig. 3, in cells treated with aspirin during the hy-

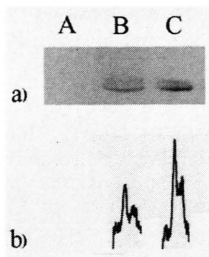


Fig. 3. Effect of aspirin and heat shock treatment on the induction of HSP70 in *Aedes albopictus* cells. Cells were maintained at 28 °C (lane A), 37 °C (lane B) or at 37 °C in the presence of aspirin (400  $\mu$ M) for 1h. (lane C). HSP70 synthesis was identified by immunoblot analysis (a) and quantified by densitometry (b) using a laser-beam densitometer (Ultrosan 2202, LKB Instruments, Bromma, Sweden).

perthermic treatment a 20% increase in the amount of HSP70 synthesis was observed.

The data described here permit us to identify  $\text{PGA}_1$  and arsenite as inducers of stress proteins in *Aedes albopictus* cells. Aspirin is an anti-inflammatory drug which inhibits the production of arachidonate metabolites via the enzyme cyclooxygenase, whose activity is a crucial mediator in many aspects of mammalian physiology. (Smith, 1989). As described in this paper an inhibitor of

prostaglandin synthesis can modulate the heat shock response, suggesting that a different product of the arachidonate cascade could be involved.

Although several data have been described concerning the role of PGs in invertebrate physiology, the mechanisms of molecular action in insects is not well understood. It is important to mention the findings of Stanley-Samuelson *et al.* (1991) who found that in tobacco hornworm *Manduca sexta*, the bacterial infection is mediated by eicosanoids. Recently we found (Barbosa and Rebello, 1995) that in *Aedes albopictus* cells,  $\text{PGA}_1$  inhibits replication of Mayaro virus (*Alphavirus* genus, *Togaviridae* family) and induce the synthesis of stress proteins. Efforts are being made in our laboratory to understand the role of heat shock proteins in the physiology of *Aedes albopictus* cells.

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