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Expression of HSP60 homologue in sheep blowfly *Lucilia cuprina* during development and heat stress

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Abstract

Among the evolutionarily most conserved molecular chaperones, members of HSP60 gene family (chaperonins) are assigned with different cellular functions, including significant role in proper folding of nascent polypeptides. We have made an attempt to specifically study the pattern of expression of HSP62, a member of HSP60 family, during development and heat stress in the sheep blowfly *Lucilia cuprina*, a pest of agricultural livestock. Studies have indicated constitutive expression of HSP62 in most of the larval and adult tissues. However, its level enhanced significantly upon heat shock in some tissues pointing its tentative role in tissue-specific thermoprotection. In situ localization of HSP60 by immunostaining or immunofluorescence in larval or adult tissues, using monoclonal anti-HSP60 antibodies, revealed its presence to be generally cytoplasmic. However, in certain polytene cell types, such as larval salivary glands, larval fat body or larval or adult Malpighian tubules, it appeared nuclear and cytoplasmic both. During oogenesis, HSP62 expression was found dynamic indicating its possible role in oocyte development. The differential levels of expression of HSP62 in different larval and adult tissues indicate their cell type specific requirement and thus, their transcription may be regulated in a tissue-specific manner. The present observations provide strong support to the role of HSP60 protein in thermotolerance, cell or tissue growth and differentiation.

Keywords: Heat shock; Heat shock proteins (HSPs); Thermotolerance; Chaperonin; HSP60; Stress proteins; Blowfly; *Lucilia cuprina*

1. Introduction

The significance of molecular chaperones (heat shock proteins (HSPs) or stress proteins) is well recognized in the progress and sustenance of normal cellular processes (Lindquist, 1986; Laskey et al., 1978). The functional significance of at least two major classes of these ubiquitous proteins, i.e., HSP70 and HSP60 chaperones, is largely correlated to their molecular structures. They help in correct folding of nascent polypeptides as well as maintenance of cellular proteins in their native (folded) functional state. Besides, they are reported to be involved in various cellular functions like intracellular translocations (Chirico et al., 1988; Deshaies et al., 1988; Gething, 1997), growth and differentiation, apoptotic cell death (Vayssier and Polla, 1998), activation of enzymes and receptors, metabolic processes and cell signalling (Ranford et al., 2000). The eukaryotic HSP60 protein has been denoted as a homologue of bacterial Cpn60, the GroEL and related to another cellular protein, TCP1 in certain eukaryotic cell types (Hemmingsen et al., 1988; McMullin and Hallberg, 1988; Ursic and Ganetzky, 1988; Cheng et al., 1989; Gao et al., 1992; Yaffe et al., 1992). The related chaperones of HSP60 family occurring in mitochondria, chloroplast and bacterial cells are termed as the "Chaperonins" (Hemmingsen et al., 1988; Ellis and Hemmingsen, 1989).

As compared to HSP70, little is known on the significance of eukaryotic HSP60. A few studies have indicated its possible role in certain cellular processes, such as germ cell differentiation, reproduction, development and thermoprotection (Kozlova et al., 1994, 1997; Meinhardt et al., 1999; Singh and Lakhotia, 2000; Timakov and Zhang, 2001). Primarily, its cellular location is mitochondria (San Martin et al., 1995; Soltys and Gupta, 1996), but a few reports indicated its presence in certain non-mitochondrial sites also, including cell nuclei, suggesting its functional relationship with cytosol and nucleus both (Sanders et al.,

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1994; Itoh et al., 1995; Soltys and Gupta, 1996; Shah et al., 1998). However, further studies in different species and tissue types are needed to substantiate the above observations. The present study is an effort to investigate some of these characteristics of HSP60 in a sheep blowfly *Lucilia cuprina*.

Our earlier study has shown that heat shock induces most of the HSPs in adult and larval tissues of the blowfly *L. cuprina* (Tiwari et al., 1995). In addition to common HSPs, two other major polypeptides of approximate molecular weights 62 and 64 kD were observed constitutively expressed in Malpighian tubules of adult fly. Only 62 kD polypeptide was found cross-reacting with HSP60 antibodies in western blots (Tiwari et al., 1995). This study is a sequel to find out (i) if the pattern of expression of HSP62kD, identified as a member of HSP60 family, is specific to adult Malpighian tubules only or is more general and (ii) to determine the pattern of expression of HSP62 in various tissue types to understand its possible functional significance during normal development and heat stress in *L. cuprina*. We found HSP62kD protein to be both constitutive as well as heat inducible in different larval and adult tissue types. The variable level of expression and the pattern of distribution of HSP60 in larval and adult tissues indicate its significant role in cell-type-specific thermoprotection as well as in the differentiation of germ line tissues, e.g., oogenesis.

2. Materials and methods

2.1. Fly culture

The laboratory stock of *L. cuprina* was maintained at $26 \pm 1\,^{\circ}C$ in the insectary and reared on sugar meal and raw goat meat.

2.2. Tissues and heat shock

From the late 3rd instar wandering stage larvae, salivary gland, brain ganglia, fat body, Malpighian tubule, midgut and hindgut were selected for analysis. Malpighian tubule, fat body, mid gut, hindgut, brain ganglion, testis and ovary were the tissues selected from the adult flies. The tissues were dissected out in Robb's minimal medium (Fristrom et al., 1973). The *in vitro* heat shock treatment was given for 1 h in a water bath set at 42 °C while control tissues were incubated at room temperature ($25 \pm 1\,^{\circ}C$).

2.3. SDS-PAGE and fluorography

Excised larval or adult tissues were radiolabelled with ^{35}S-methionine (activity: 100 μCi/ml; BRIT, BARC, Mumbai, India) during the last 30 min of heat shock treatment and at control condition. The samples were dissolved in Laemmli's sample buffer (Laemmli, 1970; also see Tiwari et al., 1995). In different experiments, equal amount (50 μg) of labelled protein, estimated by modified Bradford's method (Bradford, 1976), was loaded on a 12.5% discontinuous polyacrylamide slab gel (SDS-PAGE) for separation of polypeptides. The gel was stained with Coomassie brilliant blue for observation and monitoring of equal protein level in each lane. For fluorography, the gel was dehydrated in DMSO followed by impregnation with PPO in DMSO (Tiwari et al., 1995) and vacuum dried. The dried gel was exposed against X-ray film (Fuji) and kept at −20 °C for autoradiographic exposure.

2.4. Immunoblotting

The heat shocked and control tissues were dissolved in Laemmli's sample buffer and 80 μg protein from each sample was loaded on the standard polyacrylamide slab gels. The separated polypeptides were transferred on to Hybond-P PVDF membrane (Amersham, Hong Kong) using a semi-dry blotting apparatus (Biotech, India). The proteins were blocked in 5% fat-free milk to prevent non-specific antibody binding. The blots were washed in PBS containing 0.1% Tween-20 (Sigma, USA) and incubated in mouse anti-HSP60 (human) monoclonal antibody (Cat. No. SPA-806; StressGen, Canada) in 1:1000 dilution for 1 h, followed by subsequent washings of 30 min each in PBS with 0.2% Tween 20 (PBT). The blots were then incubated in 1:1000 dilution of anti-mouse IgG-HRP conjugate secondary antibodies (Amersham Pharmacia, Hong Kong), followed by one wash (15 min) each in PBS with 0.2% Tween-20 and 0.3% Tween-20, respectively. The HSP60 protein was detected using ECL-Plus detection system (Amersham, Hong Kong).

2.5. Squash preparation of testis

Testes were dissected out from adult flies in Robb's minimal medium and heat shocked at 42 °C for 1 h while control tissue was incubated at room temperature. For immunostaining, the method followed for permanent squash preparation was same as described in Ashburner (1989) with some modification. Tissues were fixed in 3.7% formaldehyde for 5 min, washed in 1 × PBS for 5 min and squashed on TESPA (3-Aminopropyle-triethoxysilane; Sigma, USA) coated slides. Slides were stored overnight at −20 °C in a horizontal couplin jar moistened with 3.7% formaldehyde. The cover slips were removed in chilled 50% ethanol, briefly rinsed in 100% ethanol, dried and stored at 4 °C till further processing.

2.6. Immunocytostaining

The method of immunostaining followed in this study was essentially same as described in Ashburner (1989) with some modifications. The heat shocked and control tissues were fixed in 4% paraformaldehyde (Sigma, USA) and the endogenous peroxidase activity was quenched with 0.3% hydrogen peroxide (Merck, India). The tissues were washed