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Stress protein studies in *Perna viridis*

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ABSTRACT

The cellular stress responses of most organisms in part involve the induction of a class of proteins called heat shock or stress proteins as a result of damage to existing proteins. Cellular proteins can be damaged by chemical exposures known to induce various stress proteins. Electrophoretical analysis were performed on Perna viridis hepatopancreas samples, in order to assess a possible correlation between the occurrence of 24.6 kDa protein and Polycyclic Aromatic Hydrocarbons (PAHs) exposure. A correlation between the PAHs concentrations and time of appearance was highlighted, to demonstrate this protein is synthesized in response to PAHs exposure. About the identity of 24.6 kDa protein, it could be an enzyme involved in detoxification reactions, probably Glyoxalase I.

Keywords: Mussels, Hepatopancreas, PAHs, *Perna viridis*, Electophoretic analysis

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INTRODUCTION

Protein-mediated stress responses play an important role in the protection of organisms exposed to a wide variety of chemical or physical stressors (Lindquist, 1986; Craig and Lindquist, 1988; Sanders, 1993).The proteins synthesized under stress conditions are highly conserved (Schlesinger *et al.*, 1982) and are generally induced by a sudden increase in temperature. Such proteins were termed stress proteins.

Stress proteins are involved in the protection and repair of cells following cellular damage due to exposure to a wide variety of stressors, including ultraviolet light, elevated temperatures, salinity, anoxia, pathogens, plankton blooms, heavy metals, and other contaminants such as arsenic, cyanide, pesticides, and PAHs. These proteins are highly conserved across a diverse range of phyla from bacteria to humans. The term *stress protein* does not imply that these proteins are only present in cells under stressful environmental conditions.The universality, conservation in structure, and consistency with which stress proteins are induced by a broad spectrum of stressors make them good candidates for biomonitoring of the environment (lwama *et al.*, 1998*).*

A class of proteins frequently termed stress proteins presents an early response on the intracellular level. This group actually consists of two major groups; HSPs (heat shock proteins) and GRPs (glucose regulated proteins), and these groups are both structurally and functionally closely related (Welch, 1990; Hightower, 1991). The HSPs are called so because the first known factor to induce the synthesis of those proteins was heat. In 1966, Ritossa reported that heat induced puffs in chromosomes from salivary glands of *Drosophila melanogaster* larvae, later on Tissieres *et al.*, (1974) found the synthesis of a set of new proteins in other cells of *Drosophila*. The proteins became well known as

heat shock proteins. It also became evident that when cells or whole organisms are exposed to external stress they always respond by synthesizing this small group of highly conserved proteins.

The GRPs are a group of proteins involved in glycoprotein synthesis; the synthesis of these proteins is significantly stimulated by glucose deprivation but only slightly by other stressors (Welch, 1990; Hightower, 1991).

MATERIALS AND METHODS

Small sized mussels having an average body length of 12 mm and weighing 0.35 g were selected for the test. Duplicates and controls were maintained. The mussels, *Perna viridis* were collected and transported from Sakthikulangara ($8^0$56' N, 76^0 35' E). They were cleaned off the epibiotic growths. Prior to the experiments, the mussels were acclimatized to laboratory conditions for at least 7 days in well aerated natural sea water (salinity 32 ppt, temp 28^0C, pH 7.8, dissolved oxygen >4ml/l).The animals were fed daily with artificial feeds.

The preparation of water accommodated fraction (WAFs) for use in ecotoxicity testing of crude oils involves a 20 h mixing period and at least 1 h standing for phase separation. This approach is based on the work performed by Anderson *et al.,* (1974).

The concentration of the accommodated oil was estimated in ppm basis after extraction of oil from the WAF using n-hexane.The fluorescence intensity of the hexane extract was determined against a standard of chrysene, using fluorescence spectrophotometer at wave lengths 310 nm (EX) and 360 nm (EM). The respective oil concentration in the 100% WAF was computed from the standard graph, Calculated volumes of the WAF of the Bombay High Crude oil (BHC) was then added to the test media to get the required PHC concentration.

Stress protein was analyzed by SDS-PAGE and western blotting method (Towbin *et aI.,* 1979).Hepatopancreas (20%) was homogenized in tris buffer (pH 7.5) under chilled condition and with protease inhibitor 0.1 mM PMSF (phenyl methane sulfonyl fluoride). Homogenate (n=6) were centrifuged at 5000 9 to remove the Iarge particulate matter. Supernatant was analyzed for total protein content (Lowry *et al.,* 1951). Sample buffer was immediately added to each sample and were heated to 95° C for 2 min. Sub samples of uniform protein were separated by SDS-PAGE with 12% separating and 5% stacking polyacryalmide gels using an electrode buffer (Laemmli,1970). Hela cell lysate (heat shocked, Bioreagents- LYC 101 F, Stressgen Canada) (20µg) was loaded to one lane to serve as an internal standard for blotting efficiency.Proteins were separated at 1.5 mA per well for approximately 3 hours and then electro blotted on to a total PVDF (Polyvinylidene fluoride) transfer membrane (E5781OX 1OcmSQ. USA) at 200 mA for 3 hours. After blotting, gels were stained with Coomassie blue to ensure that the complete transfer had occurred. Membranes were blocked with 3% Bovine Serum Albumin and Tris Buffer Saline (pH 7.4). Tween 20 (0.05%) in Tris Buffer Saline was used as washing solution. Primary monoclonal antibodies hsp70 developed against lobsters (1 :2000 dilution, Bioreagents-SPA 830, Stressgen, Canada) were used as probes. Horseradish peroxidase-congugated goat anti mouse IgG (1 :2000 dilution, Bioreagents-SAB-100, Stressgen, Canada) was used to detect h sp70 probes. Bound antibodies were visualized by Gel Documentation system (Syngene, UK) and were quantified using densitometry.

RESULTS

In the run of experimental rearing, mussels treated with 2 ppm of BHC, expressed a 24.6 kDa protein, never present in control mussels. This protein appeared in treated *P. viridis*, 20 days after the beginning of the exposure and disappeared 15 days after the end of depuration.

The mussels, treated with 5 ppm of WAF of BHC, expressed the 24.6 kDa protein (never present in control mussels) (Fig. 1), 15 days after the beginning of the exposure and the protein disappeared within the same time of the previous test. During the last exposure experiment, performed with 8 ppm of WAF of BHC , the protein was detected 10 days after the beginning of the treatment, and disappeared within 5 days post exposure.

From the results obtained, in *P. spp* it is clear the correlation between the exposure to WAF of PAHs and 24.6 kDa protein synthesis. In fact its presence was observed only in treated mussels.

The role of the 24.6 kDa protein, was preliminarily supposed that it might be involved in detoxification reactions or that it could be an enzyme, accumulated for indirect causes consequent PAHs poisoning.

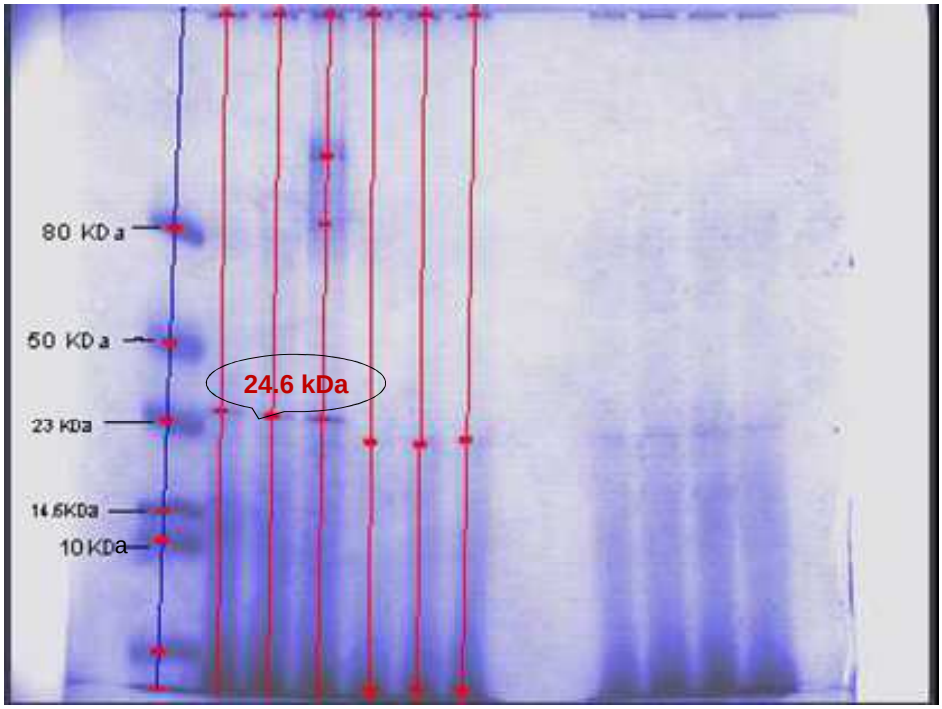

Fig.1 SDS PAGE electrophoretic pattern of exposed Perna viridis Control (lane 1); Exposed mussel that represent 24.6 kDa protein 2ppm, 5ppm and 8ppm (lane2, 3 and 4) and (lane 5,6 and 7) represents their corresponding depuration

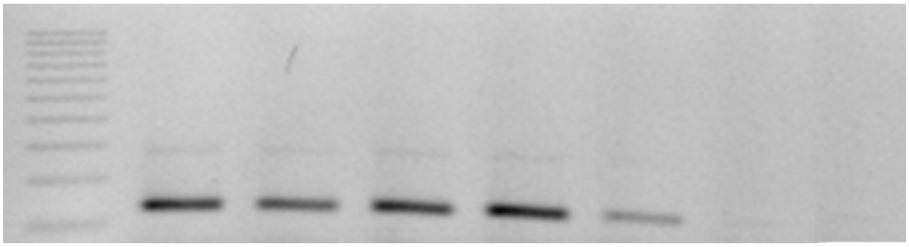

Fig.2 The Western Blot Assay control and PAH accumulated mussels and depurated states of the above. The cross-reactive antibodies are excluded and peptide specific antibodies are observed

The principal toxic effect of PAHs is due to its inhibiting effects on protein phosphatase 1 (PP1) and 2A (PP-2A) in eukaryotic cells, causing changes on protein phosphorylation state of enzymes (Bialojan and Takai, 1988).

Another system of detoxificant enzymes is glyoxalase group (Glyoxalase I, GI, and Glyoxalase II, GII), glutathione-dependent. Glutathione (GSH) is a ubiquitarian tripeptide and it is a cofactor of many enzymes catalyzing the detoxification and excretion of several toxic compounds; in particular it is a coenzyme of GI.

The GI activity is demonstrated also in *M. galloprovincialis* (Fitzpatrick *et al*., 1995).

Regoli *et al.* (1996) performed a first purification and characterization of Glyoxalase I from the digestive gland of the *Mytilus galloprovincialis* study revealed that the pure enzyme is a 48 kDa protein with an heterodimeric quaternary structure and in denaturant conditions (SDS-PAGE) it is composed of 24 and 25 kDa subunits.

Taking into account that: GI is an enzyme involved in several detoxification reactions, and 24.6 kDa protein appears after toxin (PAHs) treatment; GI consists of 2 subunits of MW very close and similar to the protein detected in the present study, and we could suppose that 24.6 kDa protein is the GI.

It is the first connection between this enzyme and the PAHs contamination. This could be a result of particular interest, because the detoxificant mechanism against PAHs and derivatives in *Perna viridis* results still unresolved.

DISCUSSION

The role of stress proteins has long been associated with protection of the cell against adverse environmental conditions, but many stress proteins are present at detectable levels in unstressed cells (Pelham, 1986). Here they play a vital role in protein homeostasis, acting as molecular chaperones directing the folding and assembly of cellular proteins (Hemmingsen *et al.*, 1988; Gething and Sambrook, 1992). When cells experience unfavourable conditions, proteins may become denatured and the synthesis of stress proteins increases. Under such conditions, stress proteins will play an additional role in the repair and protection of cellular proteins (Ellis, 1990).

Hydrogen bonding is the main stabilizing force in protein stability. Hydrophobic interactions play an important role in the thermal stability of proteins and probably in mussel proteins.. Thermal protein denaturation involves the rupture of disulfide and hydrogen bonds (Makhatadze *et al.*, 1994).

Numerous articles have reported a significant correlation between stress proteins and enhanced tolerance to stress in terms of survival and the physiological status of organisms (Krause *et al.*, 1986; Lindquist, 1986; Bosch *et al.*, 1988; Sanders 1988; Bansal *et al.*, 1991; Sanders *et al.*, 1991; Stegeman *et al.*, 1992; Krebs and Loeschcke 1994; Bond and Bradley 1995; Brown *et al.*, 1995; Tedengren *et al.*, 1999).

Reaction of mussel proteins to different types of marine pollution was well described by Porte *et al*., (2001) and Lopez *et al*., (2002).These authors have examined how some protein parameters are influenced by pollution.

A number of studies have confirmed the induction of stress proteins in mussels by elevated temperature and chemical contaminants (Sanders, 1988; Steinert and Pickwell, 1988; Veldhuizen-Tsoerkan *et al.*, 1990 ; Sanders *et al.*, 1992, 1996).

The cellular stress response is involved in protecting organisms from damage due to exposure to a variety of stressors, including temperature, heavy metals, and other xenobiotics. The stress response entails the rapid synthesis of heat shock proteins (Hsps) to protect cellular proteins against denaturation (Craig and Lindquist, 1988; Sanders 1993; Hofmann 1999).

The Western Blot Assay has been widely used to detect the presence of antibodies. In this procedure, component proteins of purified, mussel tissue are electrophoretically separated by SDS-polyacrylamide electrophoresis followed by electrotransfer to nitrocellulose sheets. Each strip serves as the solid-phase antigen for an ELISA test. The Western Blot Assay is more reliable since the cross-reactive antibodies are excluded and peptide specific antibodies are observed. However, results are yes or no and cannot be quantitated.

Indeed, this protein may represent a useful biomarker in biomonitoring studies, as an indirect index of PAHs risk.

CONCLUSION

Monitoring of the marine pollution is very important for the future of the mankind and therefore many scientists are searching for reliable biomarkers of the water pollution (Porte *et al.*, 2001; Irato *et al*., 2003). These changes in the protein profile of mussels can be used as an indicator of water pollution.

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