Biomarker for environmental stress induced aestivation

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Abstract

Factors leading to aestivation (summer sleep) invariably display features of stress and hence it is of interest to understand and elucidate the role of heat shock proteins (HSP’s) during cellular stress response in an aestivating amphibious Indian apple snail Pila globosa. Expression of Inducible HSP40 was documented in the hepatopancreatic tissue after 60 days of aestivation. The identification of Inducible HSP40 in snails exposed to long term aestivational stress could be used as a potential biomarker/bio-indicator.

Keywords: Aestivation, immunoblot, Pila globosa, HSP 40, chemiluminiscence, bio-marker.

Introduction

The phylum Mollusca is one of the largest phyla within the animal kingdom and has evolved successfully, presenting a widespread distribution, being able to survive in both aquatic and terrestrial environments. The class Gastropod possesses about 35,000 living species and 15,000 fossil records and it is one of the major classes described. The successful evolution of mollusks is a consequence of their extraordinary adaptive capacity. Most organisms are exposed to changes in their habitat environment. Such changes include temperature, humidity and vegetation. This variable environment can induce metabolic and behavioral changes in these organisms. In order to survive, these organisms migrate to other locations, change their physical characteristics or enter a hypometabolic condition (1).

The hot and dry summers of the arid & semi-arid regions of the Indian sub-continent pose an enormous challenge for many animals. The skins of amphibious and terrestrial snails are strongly water permeable and therefore easily run the risk of desiccation in hot and dry habitats. They can loose their body mass through evaporation of water. In addition, thermal death by overheating poses a danger for these animals in hot regions (2). Some of these snails, therefore, have evolved many adaptations to cope with hot and dry conditions including adaptations on morphological, physiological and behavioral levels (3-4). The major responses to temperature and other physical stress conditions are well known which involve changes in gene transcription and translation of Heat shock or stress proteins (HSPs). HSP’s are coded by a small set of heat shock and stress events requires the protein folding and maintain functional conformations. The recovery from heat shock and stress events requires the protein folding abilities of HSPs in all eukaryotes (Feder and Hoffmann 1999).

In the present study, the endemic snail, Pila globosa is chosen. Its distribution is mainly around equatorial & tropical regions of the world, is subjected to seasonal variations and in turn undergoes self-induced stress conditions of aestivation to evade long summer seasons of heat, low humidity, scarcity of green vegetation and low oxygen conditions. These environmental factors are simulated in the laboratory conditions so as to evaluate any compensatory mechanisms that snails inherently develop to overcome the adverse features of its habitat.

Materials and methods

Simulation of conditions to induce aestivation

Individuals of amphibious snail (Pila globosa) were collected from local ponds and lakes around Anantapur town of Andhra Pradesh state. The collected snails were acclimatized to laboratory conditions over a period of two-weeks by maintaining in cement water tanks. The water in the tanks was changed once every two days & water was fed with boiled spinach and Hydrilla plants. The acclimatized snails were prepared for aestivation after measuring the weight (gms) and placing them overnight on a bed of filter papers to absorb mantle cavity water. The operculum-closed snails were then kept in wooden containers 10 cms (H) x 10 cms (L) x 10 cms (B) with a bed of filter papers. Heat source was provided by keeping a 10 W bulb covered with silver foil to ensure that only heat was emanating without any light. The boxes were covered with lid having a thermometer and the temperature was maintained at 35-37°C for a period of 60 days. For sampling purposes snails were quickly sacrificed by breaking their shells and the hepatopancreas were dissected out. Samples were weighed and frozen in PBS (Phosphate buffered saline) until they were used for assessment of HSP40.

denatured (6). Environmental stress conditions such as changes in temperature (7) hypoxia (8), salinity (9), metal ion concentration (10-11) can induce the synthesis of HSPs that act to prevent protein aggregation and to maintain functional conformations. The recovery from heat shock and stress events requires the protein folding abilities of HSPs in all eukaryotes (Feder and Hoffmann 1999).

The identification of Inducible HSP40 in snails exposed to long term aestivational stress could be used as a potential biomarker/bio-indicator.
Sample preparation

100 mg of hepatopancreatic tissues were homogenized in 1 ml of ice-cold Lysis buffer (10 mM Tris, pH 7.4, 500 mM sucrose, 1 mM DTT, 100 mM NaF) containing protease inhibitors followed by mild sonication (Microson, USA) for 2 min at 15 sec interval.

Protein concentration assay

Total protein concentration in the homogenates was determined by the method described by Bradford et al. (12) using bovine serum albumin (Sigma Chemical) as a standard. For each sample 100 µl of the homogenate was used. The absorbance of the colour complex was determined at 595 nm. In the blank sample the homogenate was replaced with H2O.

SDS-PAGE

Equal amounts of protein lysates were loaded into each lane and subjected to 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDSPAGE) - for 2 hrs at 100V and stained with coomassie Brilliant blue.

Immunoblot: Total tissue lysates were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) - Immuno blot analysis with anti-HSP40 polyclonal antibody (StressGen) and Anti-Rabbit secondary antibody as described previously (13). Detection was performed by exposing the membrane to an X-ray film using enhanced chemiluminescent (ECL) reagent (Pierce) according to manufacturer’s instructions.

Two-dimensional electrophoresis

Isoelectric focusing (IEF) was performed with sonicated extracts (200 µg) by the method of Mathew et al. (14) in tube gels with a pl range of 4-10.

Results and discussion

Snails exposed to aestivation at 37°C ± 1°C and ≥ 50% relative humidity (RH) for 60 days in a simulated environment of laboratory showed up-regulation of proteins (~40 KDa) in hepatopancreatic tissue homogenates upon coomassie brilliant blue staining compared to controls (Fig. 1). Immuno blot analysis (Fig. 2) using anti-HSP40 antibody revealed the presence of two independent bands around 40KDa compared to controls where only a single 40 KDa band was observed indicating the presence of HSP40 isoforms or an inducible HSP40 upon aestivation. The above findings reveal the presence of constitutively expressed HSP40 in controls and inducible HSP40 in aestivational stress. HSP 40 is considered as co-chaperone (15) to HSP70 family of proteins which exhibit chaperone activities. Numerous studies confirmed that proteins of HSP70 family are involved in adaptations of aquatic organisms in adverse conditions (16). Results of the two-dimensional gel electrophoresis indicate two spots in aestivation exposed hepato-pancreatic tissue lysates (Fig. 3) at 40 KDa which signifies the presence of inducible HSP40 compared to a single spot in the control sample (Fig. 4).

Conclusion

HSP 40 is considered as co-chaperone (15) to HSP70 family of proteins which exhibit chaperone activities. Numerous studies confirmed that proteins of HSP70 family are involved in adaptations of aquatic organisms in adverse conditions (16). Results of the two-dimensional gel electrophoresis indicate two spots in aestivation exposed hepato-pancreatic tissue lysates (Fig. 3) at 40 KDa which signifies the presence of inducible HSP40 compared to a single spot in the control sample (Fig. 4).

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