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Induction of HSP70 by Diazinon in *Oreochromis niloticus*

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ABSTRACT

This research investigated the possibility of using heat shock protein HSP70 in fish as a biomarker to evidence chronic exposure to pollution. Equally sized male tilapia *Oreochromis niloticus* were exposed to two separate concentration of diazinon for 30 days; 0.28 mg/L (Group 1 – G1) and 1.87 mg/L (Group 2 – G2). Both groups were recovered for 7 days (Group 3 – G3 and Group 4 – G4, respectively). The four groups were compared to control fish (Group 5 – G5) of equal size. Analysis of blood samples to test HSP70 induction was done using SDS/PAGE and molecular marker ranges between 214 and 6.8kDa. HSP70 proteins (71 and 77 kDa) were induced in G1 but the induction was removed by the recovery period in G3. While 78.16 kDa was induced in G2, the induction intensity decreased in G4. These results suggest that expression of HSP70 in tilapia is sensitive to chronic exposure to diazinon contamination in aquatic ecosystems, which reflects the cellular response of fish to the stress of water pollution.

Keywords: heat-shock proteins, tilapia, toxicity, water pollution

INTRODUCTION

Most organisms residing adjacent to agricultural activities are vulnerable to pesticides, such as diazinon [O,O-diethyl O-(2- isopropyl-6-methyl-4-pyrimidinyl) phosphorothioate], which is widely used in Egypt. Although the loss of genetic diversity and biodiversity among species are potential hazards, pesticides are valuable for maintaining a healthy environment and for the welfare of humans. Heat-shock proteins (HSPs) reflect the resistance of an organism to stressful conditions, this due to their ability to react and respond to environmental and physiological stresses. This leads to denaturation of proteins in the animal's body. During disease, they may play critical roles in immune protection (Jacquier-Sarlin *et al*. 1994), antigen presentation (DeNagel and Pierce 1993) and non-specific immune responses (Guzik *et al*. 1999). HSPs refold partially unfolded proteins and detect those that are irreversibly damaged. Under non-stressed conditions, HSPs facilitate the correct folding of proteins during translation (Parsell and Lindquist 1996). Therefore, they are considered as molecular chaperones (Frydman 2001; Hartl and Hayer-Hartl 2002). Thus, from an ecological perspective, HSPs may serve as potential indicators for protein synthesis for reversible protein denaturation, and for stressed states in fish in general (Iwama *et al.* 2004). Hence, this research was conducted, in 2007, to examine hsp70 in *Oreochromis niloticus* after being exposed to diazinon.

MATERIALS AND METHODS

Diazinon was tested on 40 ± 2 g adult male *O. niloticus.* Ninety fish were divided into 3 groups, each consisting of three replicates. The first group was exposed to 0.28 mg/L (G1) while and the second group was exposed to 1.87 mg/L (G2) for 30 days, and then both were recovered for seven days (G3, G4). All groups (G1, G2, G3, and G4) were compared to untreated fish of equal size (G5). At the end of the exposure period, three replicates of blood samples were randomly taken from the fish in each aquarium and the induction of HSP70 was identified using SDS/PAGE and BioRAD molecular markers ranging between 214 and 6.8kDa. A total of 15 blood samples was prepared and injected into a PAGE gel (Laemmli 1970). A photo of the gel was captured and analyzed on a Gel documentation analyzer, Ver. 2, 2006, Elmanar Co.

RESULTS AND DISCUSSION

Examining the protein separation gel revealed the induced expression of HSP70 proteins (71 and 77 kDa) in G1, but which was totally absent in the recovery (G3). As for G2, which was exposed to a higher concentration of diazinon, 78.16 kDa proteins were induced, but the induction intensity was less in G4 (**Fig. 1**).

The induction was mostly caused by the pollution effect resulting from exposure to diazinon sublethal concentrations for 30 days which, rarely if ever, occur under natural conditions (Scholz *et al.* 2000). This may be due to the frequent water currents which resulted in dilution of the water contaminates. However, unstable temperature, hypersomatic pressure and changing salinity as well as pollution may all lead to the induction of HSP70 protein synthesis in fish (Chen *et al.* 1988 and Takeuchi *et al*. 2000). Acid or alkali treatment can have the same effect (Kim *et al.* 2003).

The induction of HSP70 proteins was probably caused by the bound FATP (fatty acid transport proteins) in the HSP70, which freely associates with nascent or misfolded peptides (open lid), causing a conformational change that activates inherent HSP70 ATPase activity. This enhances the association of HSP40 to further accelerate conversion of ATP to an ADP (closed lid) (Meyer *et al*. 2007). However, the reduction in HSP70 in G3 and G4 most likely resulted from the removal of diazinon. These results may indicate potential dysfunctions in protein folding, translocation of proteins across organellar membranes, and/or disassembly of protein complexes (Bukau 2006; Craig *et al.* 2006). They may also point out to a potential possibility of promoting mitosis of dividing cells and suppressing the reactivity of the immune system cells (Browne *et al.* 2007). However, hsp response may vary in accordance with a variety of factors related to tissue (Rabergh *et al*. 2000), stressor (Airaksinen *et al*. 2003), species (Basu *et al*. 2001; Nakano and Iwama 2002) and the developmental stage in question (Martin *et al.* 2001).

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Research Note

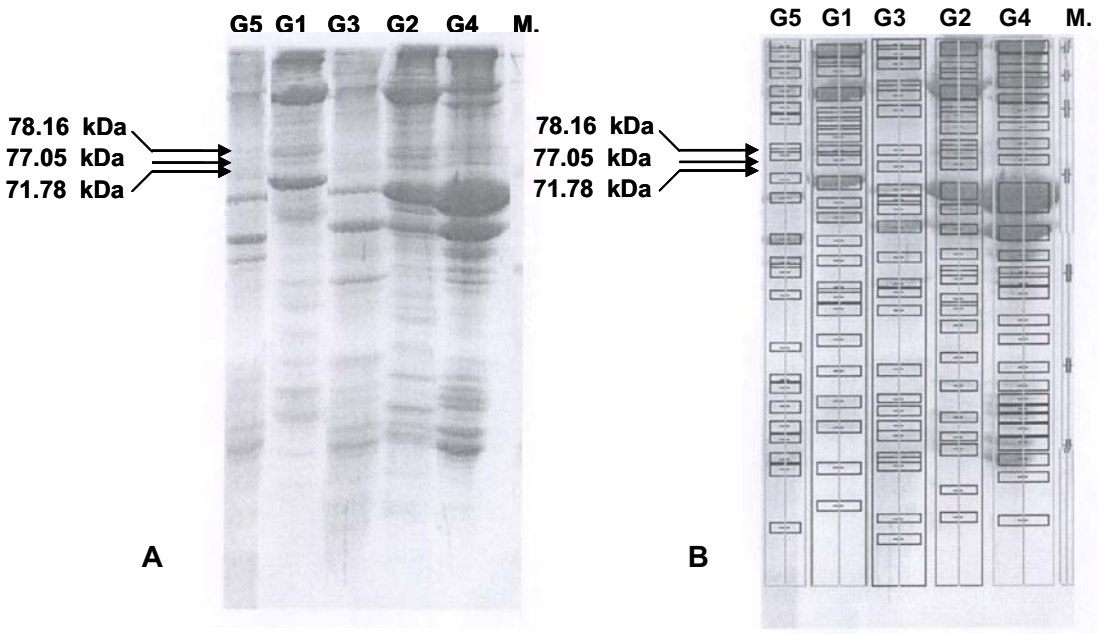

Fig. 1 Scanned separated proteins. (**A**) Stained SDS-PAGE gel. (**B**) Stained SDS-PAGE gel analyzed by GDS. Note: one of each three replicates is represented.

CONCLUSION

In conclusion, HSP70 proteins in *O. niloticus* are induced by chronic exposure to contaminated water with diazinon. These results can be applicable to use HSP70 as biomarker to reflect the cellular responses in fish to water pollution in the aquatic systems.

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