Responses of HSP Gene Expressions to Elevated Water Temperature in the Nile tilapia *Oreochromis niloticus*

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ABSTRACT : Water temperature influences on various key biological events in fish, but the internal pathway of the temperature effects are not well understood. Heat shock proteins (HSPs), known to respond in the level of cells to many environmental factors including temperature, could improve our understanding on the pathway. Some biological processes such as gonadal development and sex differentiation in the Nile tilapia *Oreochromis niloticus* is particularly sensitive to water temperature. In this study, we have investigated the expressions of HSP70 and HSP90 genes in young tilapia at an ordinary temperature (28℃) and elevated water temperature (36℃). The distribution of the expressions of HSP70 and HSP90 mRNA in this species were found to be almost ubiquitous, being detected in all tissues studied here (brain, gonad, liver and muscle), suggesting the house keeping functions of these genes. Heat shock by elevating temperature from 28℃ to 36℃ significantly increased the expression of HSP70 mRNA in the gonad, liver and muscle for several hours ($P<0.05$) (brain tissue was not examined for this). The increased level of HSP70 gene expression recovered to the level at control temperature (28℃) when fish were kept continuously at high temperature (36℃) for 24 hours. Contrary to this, expression of HSP90 mRNA did not show significant increase in the gonad and muscle by the same heat shock ($P>0.05$), except in the liver where the expression of HSP90 mRNA increased continuously for 24 hours at 36℃. The results obtained in this study suggest that response to temperature change in different tissue or organ may utilize different heat shock proteins, and that HSP70 may have some importance in temperature-sensitive gonadal event in the Nile tilapia.

Key words : Heat shock protein, HSP70, HSP90, Gene expression, Water temperature, Gonad, Liver, Muscle, Nile tilapia, *Oreochromis niloticus*.

INTRODUCTION

The body temperature of fish, as a poikilotherm, changes along with the water temperature of the surroundings. Thus, daily changes and seasonal changes of water temperature have powerful and adverse effects on many important biological processes including development, growth, reproduction and resistance to disease. Aquaculturists have identified optimal temperatures for the farming of various fish species. However, the routes of the temperature action on many biological processes in fish are not well understood.

Heat Shock Proteins (HSPs) were found in fruit fly exposed to heat shock for the first time in 1962 (Ritossa, 1962), since then, the presence of these protein have been confirmed in a variety of organisms such as embryonic cells of chicken (Kelly & Schlesinger, 1978), *E. coli* (Lemeux et al., 1978), yeast (McAlister & Finkelstein, 1980), plant (Barnett et al., 1980), mammals (Hunt & Morimoto, 1985; Hunt & Calderwood, 1990), and fish (Oda et al., 1991). These proteins are well conserved in the evolution and known to be produced within the cells in response to various environmental stresses including temperature (Iwama et al., 1998). They are expressed in the normal cell without stress and carry out many functions associated with protein metabolism. However, the level of expression increases 10-20 folds under stress to regulate energy metabolism and to re-establish homeostasis (Basu et al., 2002).

HSPs are considered to be potential biomarkers in fish since they respond to many biotic and abiotic stressors. Of

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the heat shock proteins, HSP70 has been best implicated as a potential biomarker since the elevation of its synthesis under stressful conditions was repeatedly observed in various fish species (Iwama et al., 1998). Synthesis of HSP90, another HSP, was also found to be enhanced at elevated temperatures in some fish species (Dietz & Somero, 1992), but the response appeared to be tissue specific. These proteins could be particularly useful in studying temperature-associated biological events.

Nile tilapia, *Orochromis niloticus*, is one of most important aquaculture species that is cultured worldwide. This species is also widely used as an experimental fish for the study of lower vertebrates due to the strong resistance to disease and convenience of rearing and breeding in a laboratory. The sex of Nile tilapia is unstable and affected by many non-genetic factors including water temperature (Kwon et al., 2002). In addition, spawning activity of this species is largely dependant on surrounding temperature. These informations together propose a possible relationship between HSPs and temperature-affected sexual events in this species. As the first step to clarify such relationship, we investigated the expression patterns of HSP70 and HSP90 genes in different tissues of tilapia either at an ordinary temperature or high temperature.

MATERIALS AND METHODS

Young Nile tilapia (10-13 ㎝ in total length), produced in fish rearing facilities in Sunmoon University (Asan, Chungnam, Korea), were divided into two groups and separately accommodated at two different temperatures (ordinary temperature: 28℃ or high temperature: 36℃). The temperature of 34-36℃ is considered to be the threshold of normal development in this species (Kwon et al., 2002). Water temperature for both groups was initially 28℃, but, for the exposure to 36℃, water temperature in one of the two groups was gradually elevated during the first 1 hour from 28℃ to 36℃ by using an electric heater. At 1, 3, 5 and 24 hours after the beginning of the experiment, three fish were sampled from the respective temperature group (24 fish in total: 3 fish at each×4 times of sampling×2 different temperature groups). The gonad, liver and muscle tissues were removed from the sampled fish, snap frozen on dry ice and kept at −70℃ until analysis. Apart from the temperature experiment, some immature tilapia being raised at the ordinary temperature were sacrificed and their brain, gonad, liver and muscle tissues were also removed to investigate the tissue distributions of HSP gene expressions.

Total RNA from the sampled tissues were prepared using TRI reagent (MRC Inc.) and each RNA (2 ㎍) was reverse transcribed into cDNA *in vitro* using M-MLV reverse transcriptase (Promega) following the standard procedure. The resultant cDNA (1 ㎕) was used as a template for subsequent PCR. Primers for the PCR of HSP70 and HSP90 genes (Table 1) were designed based on the partial sequences obtained from our preliminary study (Kim & Kwon, 2007; Kim et al., 2007). As a control gene, β-actin gene primers were synthesized by following the primer sequences utilized in a previous study (Kwon et al., 2001). The PCR conditions for HSP70 gene were 1 cycle at 94℃ for 2 minutes, 56℃ for 1 minute, 72℃ for 1 minute; 30 cycles (except for the gonad - 24 cycles) at 94℃ for 1 minute, 60℃ for 30 seconds, 72℃ for 1 minute; 1 cycle at 72℃ for 6 minutes. The PCR conditions for HSP90 gene were 1 cycle at 94℃ for 2 minutes, 56℃ for

Table 1. Primers used for reverse transcription-polymerase chain reaction (RT-PCR) to study the effect of elevating temperature on the expression of HSP70 and HSP90 genes

Genes	Primer sequences (5'→3')	Product sizes
HSP70	Fw: AGATGTCTGCAGCTAAAGGT Re: CGCTGGGAGTCGTTGAAGTA	472
HSP90	Fw: TTTGCTGAACTGGGTGAGGA Re: AGAGACCAGGGTCTTGCCAT	340
β-actin	Fw: AATCGTGCGTGACATCAAGG Fe: AGTATTTACGCTCAGGTGGG	392

Fw: forward primer, Re: reverse primer.

1 minute, 72℃ for 1 minute; 24 cycles at 94℃ for 1 minute, 56℃ for 30 seconds, 72℃ for 1 minute; 1 cycle at 72℃ for 6 minutes. The PCR conditions for β-actin gene were 1 cycle at 94℃ for 2 minutes, 57℃ for 1 minute, 72℃ for 1 minute; 30 cycles at 94℃ for 1 minute, 57℃ for 30 seconds, 72℃ for 1 minute; 1 cycle at 72℃ for 6 minutes. Reagents for the PCR were purchased from Sungenetics Inc.

PCR products were run in a 1% agarose gel in 1×TBE buffer, visualized on a UV transilluminator (with gel documentation system, Kodak 1D) and digitalized by measuring the intensities of the visualized DNA bands. The relative expression level of HSP genes to that of β-actin was calculated and used to compare the level between 28℃ and 36℃. The data were shown as mean±SEM (n=3). Statistical differences of relative HSP mRNA expression to β-actin mRNA between the two temperatures were determined by *t*-test ($P<0.05$).

RESULTS

1. Tissue Ditribution of HSP70 and HSP90 Gene Expressions

Tissue distribution of HSP70 and HSP90 mRNA was investigated in the brain, gonad, liver and muscle of immature Nile tilapia using reverse transcription-PCR (Fig. 1). The expressions of both HSP genes in this species were found to be almost ubiquitous, being detected in all tissues studied here (brain, gonad, liver and muscle), suggesting the house keeping functions of these genes. The strongest expression was observed in the gonad for both HSP genes and the weakest in the liver for HSP70. The expression of HSP90 gene was relatively higher than that of HSP70 gene in all tissues studied here. In Fig. 1, HSP70 gene was amplified 31 cycles but HSP90 gene was only 25 cycles.

2. Responses of HSP70 and HSP90 Gene Expressions to Elevated Water Temperature

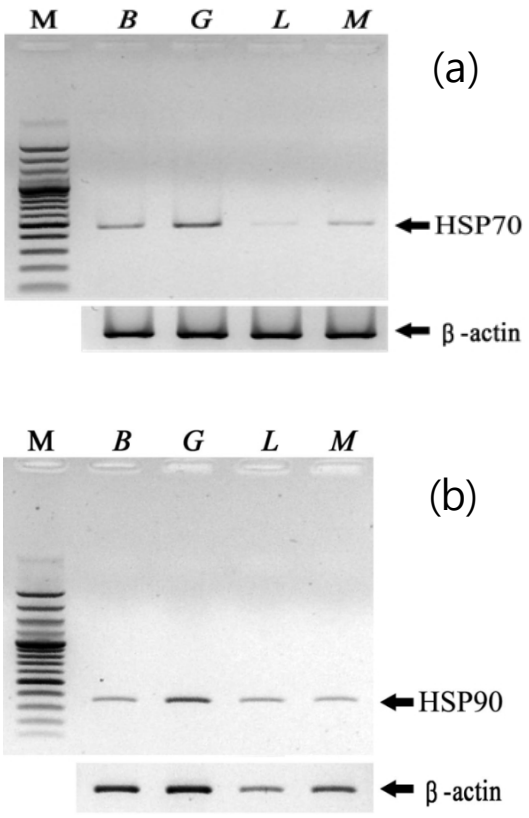

Fig. 1. Tissue distribution of HSP70 and HSP90 gene expressions at ordinary temperature (28℃) in the brain (*B*), gonad (*G*), liver (*L*) and muscle (*M*) of tilapia *Oreochromis niloticus*. M: size marker.

Heat shock by elevating temperature from 28℃ to 36℃ significantly increased the expression of HSP70 gene in the gonad, liver and muscle at 1 and 3 hours after the beginning of the experiment ($P<0.05$) (Fig. 2). The level of HSP70 gene expression at 36℃ became no more significantly different from the expression level at control temperature (28℃) at 24 hours after the beginning of the experiment ($P>0.05$). The increased level by heat shock lasted longer in the liver and muscle than in the gonad.

Contrary to this, expression of HSP90 gene did not show significant increase in the gonad and muscle by the same heat shock ($P>0.05$) with the exception of the liver where the expression of HSP90 gene significantly increased ($P<0.05$) and stayed at high levels continuously when fish were kept at 36℃ for 24 hours (Fig. 3). No mortality has

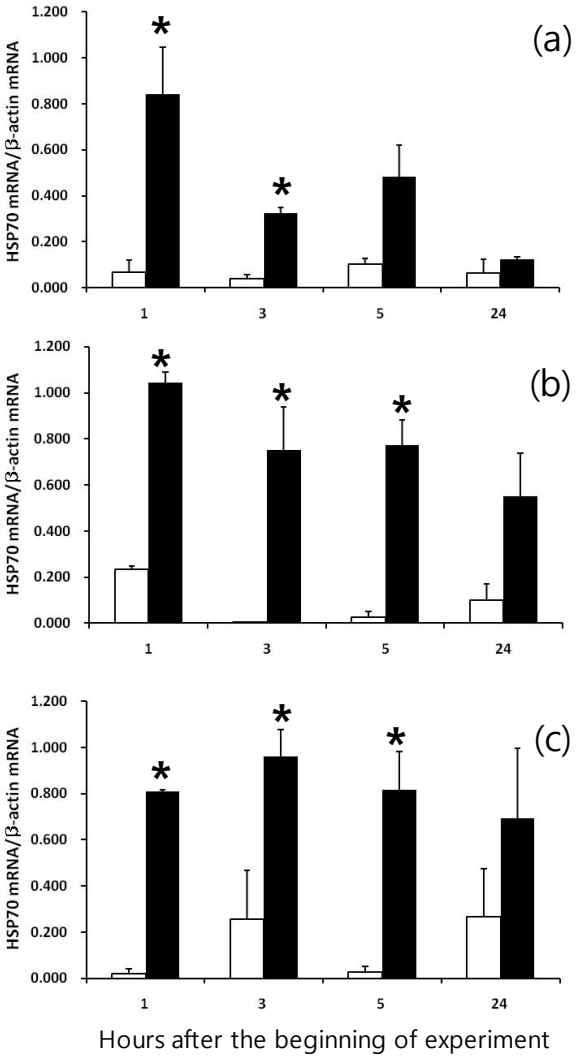

Fig. 2. Differences of HSP70 gene expression in the gonad (a), liver (b) and muscle (c) at 28℃ (white column) and 36℃ (black column). The expressions of HSP70 gene and β-actin gene (as a control) were examined by using semi-quantitative RT-PCR analysis (in triplicates). PCR products were run in a 1% agarose gel. * indicates significant difference of HSP70 gene expression between 28℃ and 36℃.

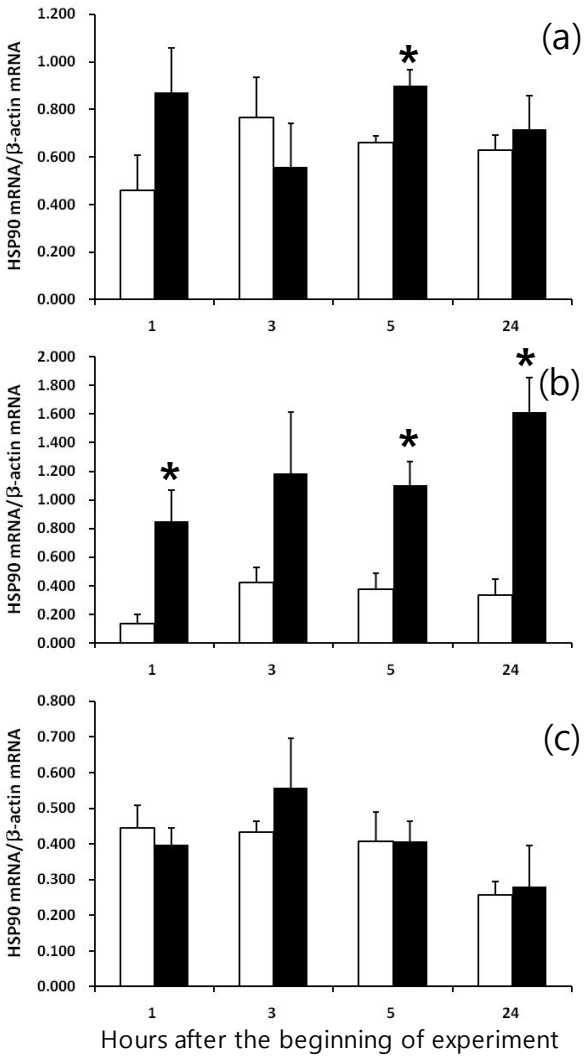

Fig. 3. Differences of HSP90 gene expression in the gonad (a), liver (b) and muscle (c) at 28℃ (white column) and 36℃ (black column). The expressions of HSP90 gene and β-actin gene (as a control) were examined by using semi-quantitative RT-PCR analysis (in triplicates). PCR products were run in a 1% agarose gel. * indicates significant difference of HSP90 gene expression between 28℃ and 36℃.

occurred during the elevation of water temperature.

DISCUSSION

HSP70 and HSP90 mRNA were ubiquitously expressed in all tissues studied here in young Nile tilapia. However, HSP90 mRNA was far more abundant than HSP70 mRNA when fish were not subjected to thermal stress. PCR product of HSP90 gene amplified 31 cycles were comparable to that of HSP70 gene amplified 25 cycles in this study. HSPs are known to have a variety of constitutive and house keeping functions in various aspects of protein

metabolism in the unstressed cell (Fink & Goto, 1990). The results from the present study imply that HSP90 may have more significance in house keeping role than HSP70 has.

HSP70 appears to be more important in protecting cells under unusual circumstances caused by environmental stress including heat shock. In our study, heat shock by elevating temperature from 28℃ to 36℃ significantly increased the expression of HSP70 gene in the gonad, liver and muscle for several hours. The expression level at the time of 1 hour after the beginning of heat shock was 4-40 folds greater than the level at control temperature. Similar results were previously reported from a work with Mozambique tilapia which is closely related species to Nile tilapia (Molina et al., 2000). In this previous work, the authors found the presence of HSP70 mRNA in various tissues only after heat shock. On the other hand, in this study, expression of HSP90 gene did not show significant increase in the gonad and muscle by the same heat shock, except in the liver where the expression of HSP90 increased continuously for 24 hours at 36℃.

The inducible feature of HSP70 by heat shock was well studied and explained by the absence of introns in the gene structure (Basu et al., 2002; Molina et al., 2000). Without introns, the mRNA is quickly translated into nascent protein within minutes following exposure to thermal stress. In our study, the increased level of HSP70 gene expression recovered to the level at control temperature within 24 hours even though fish were kept continuously at high temperature. Supporting this finding, Basu et al. (2001) demonstrated that cortisol significantly suppressed the heat stress-induced level of HSP70. Based on their explanation, HSP70 takes part in forming a glucocorticoid receptor complex. When cortisol level increases, the cortisol bind to the receptor complex, displacing HSP70. This free HSP70 may act through a negative feedback loop to inhibit further HSP gene transcription and translation. These findings together suggest that the inducible HSP70 might be responsible for the fast protection to stressors until the slow protection system (elevation of cortisol and glucose level, and also other HSP-possibly HSP90 in the liver) starts to work.

Among the tissues studied in this study, the gonad showed the highest level of HSP70 gene expression at both ordinary temperature and elevated temperature (cDNA from the gonad for HSP70 was amplified only 25 cycles unlike 31 cycles for the cDNA from other tissues). This implies that the gonad and gonadal events in this species are highly sensitive to surrounding water temperature and HSP70 might be associated with some important temperature-dependant sexual and gonadal processes. In support of this, Akatsuka et al. (2004) found a higher transcription activity of HSP70 binding protein in the gonadal area than in other tissues during sex differentiation in frog embryos and activation of the protein *in vitro* by estrogen treatment, signifying the importance of HSP70 in the process of sex differentiation.

Taken together, the results obtained in this study suggest that responses to temperature change in different tissues or organs may utilize different heat shock proteins, and that HSP70 may have some importance in temperature-sensitive gonadal event in the Nile tilapia. Further studies on HSPs in this species should extend to various tissues including the brain and gill, and pay more attention to the possible link between sex steroids and HSP.

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REFERENCES

Akatsuka N, Kobayashi H, Watanabe E, Iino T, Miyashita K, Miyata S (2004) Analysis of genes related to expression of aromatase and estradiol-regulated genes during sex differentiation in *Xenopus* embryos. Gen Comp Endocrinol

136:382-388.

Barnett T, Altschuler M, McDaniel CN, Mascarenhas JP (1980) Heat shock induced proteins in plant cells. Dev Genet 1:331-340.

Basu N, Nakano T, Grau EG, Iwama GK (2001) The effects of cortisol on heat shock protein 70 levels in two fish species. Gen Comp Endocrinol 124:97-105.

Basu N, Todgham AE, Ackerman PA, Bibeau MR, Nakano K, Schulte PM, Iwama GK (2002) Heat shock protein genes and their functional significance in fish. Gene 295:173-183.

Dietz TJ, Somero GN (1992) The threshold induction temperature of the 90kDa heat shock protein is subjected to acclimatization in eurythermal goby fishes (genus *Gillichthys*). Proc Natl Acad Sci USA 89:3389-3393.

Fink AL, Goto Y (1998) Molecular Chaperones in the Life Cycle of Proteins: Structure, Function, and Mode of Action, Marcel Dekker, New York.

Hunt C, Calderwood S (1990) Characterization and sequence of a mouse hsp70 gene and its expression in mouse cell line. Gene 87:199-204.

Hunt C, Morimoto RI (1985) Conserved features of eukaryotic HSP70 genes revealed by comparison with the nucleotide sequence of human HSP70. Proc Natl Acad Sci USA 82:6455-6459.

Iwama GK, Thomas PT, Forsyth RB, Vijayan MM (1998) Heat shock protein expression in fish. Rev Fish Biol Fish 8:35-56.

Kelly PM, Schlesinger MJ (1978) The effect of amino acid analogues and heat shock on gene expression in chicken embryo fibroblasts. Cell 15:1277-1286.

Kim J, Kim SH, Kwon JY (2007) Molecular cloning of HSP90 gene and tissue distribution of its expression in tilapia *Oreochromis niloticus*. In: Proc. 24th Scientific Conference of Korean Society of Developmental Biology, Busan, Korea, 13 Jun, 2007, pp 96-97.

Kim J, Kwon JY (2007) Changes of HSP70 gene expression in response to elevated water temperature in tilapia *Oreochromis niloticus*. In: Proc. 24th Scientific Conference of Korean Society of Developmental Biology, Busan, Korea, 13 Jun, 2007, pp 98-99.

Kwon JY, McAndrew BJ, Penman DJ (2001) Cloning of brain aromatase gene and expression of brain and ovarian aromatase genes during sexual differentiation in genetic male and female Nile tilapia *Oreochromis niloticus*. Mol Reprod Dev 59:359-370.

Kwon JY, McAndrew BJ, Penman DJ (2002) Treatment with an aromatase inhibitor suppresses high-temperature feminization of genetic male (YY) Nile tilapia. J Fish Biol 60:625-636.

Lemeux PG, Herendeen SL, Block PL, Neidhardt FC (1978) Transient rates of synthesis of individual polypeptides in *E. coli* following temperature shifts. Cell 13:427-434.

McAlister L, Finkelstein DB (1980) Heat shock proteins and thermal resistance in yeast. Biochem Biophys Res Commun 93:819-824.

Molina A, Biemar F, Müller F, Iyengar A, Prunet P, Maclean N, Martial JA, Muller M (2000) Cloning and expression analysis of an inducible HSP70 gene from tilapia fish. FEBS Letters 474:5-10.

Oda D, Mitani H, Naruse K, Shima A (1991) Synthesis of heat shock proteins in the isolated fin of the medaka, *Oryzias latipes*, acclimated to various temperatures. Comp Biochem Physiol 98B:587-591.

Ritossa FM (1962) A new puffing pattern induced by a temperature shock and DNP in *Drosophila*. Experimentia 18:571-573.

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