HSP60 expression profile under different extreme temperature stress in albino northern snakehead, *Channa argus*

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Abstract

The great albino northern snakehead, *Channa argus*, is one of the most important economical fish in China. In the present study, cDNA encoding heat shock protein 60 (HSP60) was cloned and characterized. The cDNA was 2462 bp, containing an open reading frame (ORF) encoding a 575-amino-acids polypeptide of 61.10 kDa (theoretical isoelectric point [pI]: 5.66). BLAST analysis showed that AcaHSP60 was highly similar with other HSP60s, and three conserved amino acid blocks and characteristic motifs or domains defined as HSP60 protein family signatures. Genomic DNA analysis showed that AcaHSP60 had ten exons in the coding region (from 94 to 336 bp). Changes in AcaHSP60 gene expression profiles in albino *C. argus* experimentally exposed to different temperature stress (8.5, 26, and 37 °C) was investigated. Quantitative real-time PCR and western blot analysis revealed that tissue-specific AcaHSP60 expressions were in the spleen, muscle, liver, kidney, heart and brain. Expression was highly significantly stimulated after heat shock (37 °C), but showed no significant differences after cold treatment (8.5 °C) except in the brain. In summary, these results showed that AcaHSP60 was significantly tissue specific and indicate that AcaHSP60 expression might be sensitive to thermal resistance in albino *C. argus*.

Keywords Albino *Channa argus* · Heat shock protein 60 · Temperature stress · Gene expression

Introduction

Heat shock proteins (HSPs) are generally responsible for preventing damage to proteins in response to high heat

(Habich and Burkart 2007; Itoh et al. 2002). According to their molecular mass, HSPs are classified into six major families: small HSPs, HSP40, HSP60, HSP70, HSP90, and HSP110 (Quan et al. 2017). Among them, HSP60 plays an extremely important role in cell protection (Eggert-Kruse et al. 2015). For this reason, we thought that the temperature resistance of introduction, domestication, and the vulnerability, etc., of albino *C. argus* might be closely related with the heat shock proteins.

The northern snakehead (Perciformes, Channoidei, Channidae) is widely distributed in Asia and Africa, which is considered as an economically valuable fish (Cheng and Zheng 1987; Courtenay and Williams 2004) and has potential medical and pharmaceutical properties (Liu et al. 2000). The normal northern snakehead *Channa argus* is widely cultivated in the coldest north and hottest south of China, which the survival temperature is 0–41 °C (Liu 1999), and has become one of the most promising aquaculture species in the early twenty-first century. Surprisingly, one albino version was found in the Jialing River in Sichuan (105.05E, 29.58N), and has gradually become a more valuable and superior variety for breeding (Su and Xiong 2011; Zhou et al. 2017a).

Aiguo Zhou and Shaolin Xie contributed equally to this work.

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Similarly, we have introduced the albino *C. argus* to southern China for breeding. There they must contend with a temperature change in addition to the environmental issue of climate change (IPCC 2007; Mohanty and Mohanty 2009). However, both the normal and albino snakehead fish are experiencing disease and increased mortality. The fish surface is prone to oval ulcers characterized by scale loss and skin and muscle rot in serious cases (Cheng et al. 1990; Johnson et al. 2003; Santoro 2000). Therefore, the introduction and culture of albino *C. argus* face some challenges due to its unique living environment. Here, we tested different temperatures and probed the genetic responses of AcaHSP60 in albino northern snakehead. Generally, our current findings indicated that *C. argus* has a strong tolerance toward both high and low temperatures, which may be related to proteins that respond to temperature stress. In this study, the expression of HSP60 gene in the different tissues (spleen, muscle, liver, kidney, heart, and brain) of albino *C. argus* was analyzed by quantitative real-time PCR (qRT-PCR). What surprised us is that the AcaHSP60 expression might be sensitive to thermal resistance, but not for low temperature. The results will contribute to the understanding of the response mechanism of albino *C. argus* when it is introduced to the south or north of China and the whole world, as well the mechanism of the study of HSP60 gene after the change of fish survival temperature.

Materials and methods

Animals

Apparently healthy, wild albino *C. argus* (length 15.28–17.32 cm, weight 55.92–70.29 g) were collected from the Jialing River, Neijiang city (105.05E, 29.58N), Sichuan province, China. They were acclimatized in an aquarium (100 × 80 × 60 cm³) at 26 ± 1 °C under a 12 h:12 h (light: dark cycle) for at least 15 days before temperature treatment, and the survival rate was 100%. For temperature treatment, six test fish were exposed to each desired temperature (8.5, 26, and 37 °C) for 2 h, and then returned to the acclimation temperature (26 °C) for 1 h (Nakano and Iwama, 2002). The spleen, muscle, liver, kidney, heart, and brain tissues of each fish were dissected, flash-frozen in liquid nitrogen, and stored at –80 °C until RNA extraction.

Full-length cDNA cloning and sequencing of AcaHSP60

Total RNA was extracted from the spleens of albino *C. argus* using TRIzol reagent and treated with RNase-free DNase I (both from TaKaRa, Japan). RNA quality was assessed by electrophoresis on a 1.5% agarose gel. The concentration and purity were determined at optical

density (OD)_{260/280} with a Nanodrop ND-2000 spectrophotometer (Thermo Electron Corporation, USA). cDNA synthesis was carried out using PrimeScript® RT reagent Kit with gDNA Eraser (TaKaRa) according to the manufacturer's instructions. The cDNA products were stored at –20 °C for later use. The first strand of cDNA was used as a template to amplify the central fragments of AcaHSP60 using gene-specific primers (AcaHSP60F and AcaHSP60R), which were designed according to the sequences of *C. striata*, *Epinephelus coioides*, and *Danio rerio*. The full-length sequences of the AcaHSP60 cDNA were obtained by RACE (rapid amplification of the cDNA ends). 5'-RACE and 3'-RACE were performed using the 5'-full RACE core set and 3'-full RACE core set (TaKaRa), respectively. Genomic DNA was extracted from the muscle of albino *C. argus* by using a Marine Animal Tissue Genomic DNA Extraction Kit (Tiangen Biotech (Beijing) Co., Ltd.). An mRNA-to-genomic alignment program (<http://www.ncbi.nlm.nih.gov/spidey/index.html>) was used to determine the exon-intron structure based on the cDNA and genomic DNA sequence of AcaHSP60 gene. The primers are shown in supplementary Table 1.

Molecular characteristic and phylogenetic analysis of AcaHSP60 cDNA

Homologous sequences of AcaHSP60 cDNA were searched in GenBank using the searching tool Blastn from the National Center for Biotechnology Information (NCBI) website. The amino acid sequence of AcaHSP60 gene was predicted and analyzed using Lasergene software and aligned with other homologous genes using the Clustal W program and DNASTar software. The open reading frame (ORF) of the AcaHSP60 gene was deduced by the means of ORF Finder in NCBI (<http://www.ncbi.nlm.nih.gov/gorf/gorf.html>). Then, a phylogenetic tree was constructed by the Neighbor-Joining analysis with 1000 bootstrap replicates using MEGA 6.0 software. Protein structure of AcaHSP60 was predicted using Swiss-model (an online platform for protein structure and function predictions, <http://swissmodel.expasy.org/>).

Relative qRT-PCR

We analyzed AcaHSP60 gene expression levels in multiple tissues (spleen, muscle, liver, kidney, heart, and brain) under different treatments with fluorescent qPCR using the Bestar® SybrGreen qPCR mastermix (DBI, Germany). The qPCR primers for the AcaHSP60 gene were designed (Table 1), and their specificities were examined by conventional PCR and melting curve analyses. β -actin gene expression was used as the internal control (Jia et al. 2010). Real-time quantitative PCR was

Fig. 1 Genomic DNA structures of AcaHSP60. The boxes represented exons. Fold lines or straight lines represented introns. 5'- and 3'-UTRs were shown as dashed lines

performed on an iQ5 Real-time PCR instrument (Bio-Rad, USA). Relative expression of albino *C. argus* AcaHSP60 mRNA was determined using the $2^{-\Delta\Delta C_t}$ method (Livak and Schmittgen 2001). The amplification efficiency (E) value of qPCRactinF/qPCRactinR and qPCRacaHSP60F/qPCRacaHSP-60R was 99.1 and 95.9%, which was calculated as the curve of C_t (Log (cDNA dilution)). The value indicated n -fold difference relative to the blank group, which was used as the reference sample and calibrator, respectively.

Statistical analysis

Expression fold change data were logarithmic transformed before performing one-way analysis of variance (one-way ANOVA) using SPSS 17.0 (SPSS Inc., USA). When overall differences were significant, Tukey's test was conducted to compare the means between individual treatments. Differences were considered significant and highly significant at $p < 0.05$ and $p < 0.01$, respectively.

Fig. 2 Phylogenetic relationship between the amino acid sequences of AcaHSP60 and 35 available HSP60s

Results and discussion

Sequence analysis of AcaHSP60

Using RACE and nested PCR approaches, a full-length cDNA (GenBank accession no. KT819287) and genomic DNA (GenBank accession no. KU883612) of the albino *C. argus* HSP60 (AcaHSP60) was obtained. The AcaHSP60 cDNA consisted of 2452 bp, including an ORF of 1728 bp encoding a protein of 575 amino acids with predicted molecular mass of 61.10 kDa and a theoretical isoelectric point (pI) of 5.66, a 5'-terminal untranslated region (UTR) of 104 bp, a 3'-terminal UTR of

467 bp with a canonical polyadenylation signal sequence AATAAA and a poly (A) tail. The deduced amino acid sequence of AcaHSP60 contains several conserved motifs or domains (a mitochondrial presequence, a HSP60 mitochondrial family characteristic sequence AAVEEGIVPG GG at the 430–441 position, a characteristic HSP60 family signature, an ATP binding site, and a 3' terminal typical GGM repeat motif). Meanwhile, it showed many potential functional sites, which allowed the signals transferred from cell membrane to the nucleus, and may also be involved in phosphorylation and dephosphorylation of cellular signaling (Ricci et al. 2016). The genomic DNA sequences of AcaHsp60 were amplified and confirmed (Fig. 1).

Fig. 3 The expression of HSP60 mRNA relative to β -actin analyzed by qRT-PCR in albino *C. argus* after different temperature exposure treatments. ((a) Spleen, (b) Muscle, (c) Liver, (d) Kindey, (e) Heart, (f) Brain). The results were expressed as mean $\pm$ standard error (shown as bars) after normalizing the values to β -actin expression (internal control). Significant differences between the treatment and control groups were indicated (* $p < 0.05$ and ** $p < 0.01$) based on the Tukey HSD test

Evolution of AcaHSP60

Phylogenetic relationship between the amino acid sequences of AcaHSP60 and 35 available HSP60s was constructed using the MEGA 6.0 programs (Fig. 2). The phylogenetic tree showed that it displayed extremely high similarity to HSP60s of the osteichthyes such as *C. striata* (96.8%), *E. akaara* (94.1%), *Paralichthys olivaceus* (93.7%), *Misgurnus anguillicaudatus* (90.7%), and *Dania rerio* (90.2%); meanwhile, a relatively high similarities were found in bacteria (41.8–49.1%) compared with AcaHSP90 (Zhou et al. 2017b). Overall, the multiple sequence alignment showed that the regions of HSP60 family signatures were highly conserved. All the selected species were clustered into two major branches, and the bacteria clustered in a separate clade. Meanwhile, all the vertebrates were clustered together and formed two branches. According to the distance from the root of trees, the evolution sequence of the three groups was vertebrates, mollusks, crustaceans, insects, and bacteria, which conformed to the animal life tree (Adoutte et al. 2000). Taken together, the sequence alignment, structure comparison, and phylogenetic analysis indicated that AcaHSP60 is a cytosolic member of HSP60 family, which were known as ubiquitous and highly conserved molecular chaperones.

Gene expression analysis

qRT-PCR with AcaHSP60 gene-specific primers (qAcaHSP60F/R) was employed to measure AcaHSP60 transcripts in different tissues in the same treatment groups. The β -actin gene was used as an internal control (Jia et al. 2010). The relative expression levels of AcaHSP60 in albino *C. argus* tissues following different temperature treatments were presented in Fig. 3. The AcaHSP60 expressions were significantly increased in all the five tissues after heat shock (37 °C). Similar results were also reported in the previous studies conducted using channel catfish (Xie et al. 2015), *Siniperca chuatsi* (Wang et al. 2017), *Oncorhynchus mykiss* (Shi et al. 2015), *Apostichopus japonicus* (Xu et al. 2014a), and *Penaeus monodon* (Shi et al. 2016). Surprisingly, the brain showed a significantly decreased after cold treatment (8.5 °C), while other tissues had no significant difference. These results were in agreement with HSP60 expression levels in *Pomacea canaliculata* (Xu et al. 2014b). The AcaHSP60 expression also showed tissue specificity for the temperature treatment.

In summary, rising temperature, alternation of water environment, and other uncontrollable factors are the major issue when albino *C. argus* were introduced into South China, and these issues can cause huge survival stress and disease outbreak (Zhou et al. 2017b). Surprisingly, the heat shock protein plays an important role in the process of introduction and breeding, especially for the temperature changes. Meanwhile, our current findings indicated that *C. argus* had

a strong tolerance toward both high and low temperatures, which may be related to proteins that respond to temperature stress. The results also indicated that the AcaHSP60 plays an important role in biological life activities, which also laid a theoretical foundation for further research on the expression of HSP60 protein in vitro, the validation of physiological function, and its regulation mechanism.

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Compliance with ethical standards All of the procedures and animal handling were performed in accordance with the guide for the Chinese Association for Laboratory Animal Sciences. Approval of the study was obtained from the Animal Ethics Committee of South China Agricultural University.

The authors alone are responsible for the content and writing of this article.

Conflict of interest The authors declare that they have no conflicts of interest.

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