Comparative Biochemistry and Physiology, Part A 156 (2010) 262–268

Contents lists available at ScienceDirect

Comparative Biochemistry and Physiology, Part A

journal homepage: www.elsevier.com/locate/cbpa

Profiles of antioxidant gene expression and physiological changes by thermal and hypoosmotic stresses in black porgy (*Acanthopagrus schlegeli*)

Kwang Wook An [a], Na Na Kim [a], Hyun Suk Shin [a], Gyung-Suk Kil [b], Cheol Young Choi [a,*]

[a] Division of Marine Environment & Bioscience, Korea Maritime University, Busan 606-791, Republic of Korea
[b] Division of Electrical & Electronic Engineering, Korea Maritime University, Busan 606-791, Republic of Korea

ARTICLE INFO

Article history:
Received 30 December 2009
Received in revised form 11 February 2010
Accepted 11 February 2010
Available online 19 February 2010

Keywords:
Catalase
Glutathione peroxidase
Hypoosmotic stress
Oxidative stress
Superoxide dismutase
Thermal stress

ABSTRACT

We determined oxidative stress by measuring the expression and activity of 3 antioxidant enzymes [Cu/Zn-superoxide dismutase (Cu/Zn-SOD), catalase (CAT) and glutathione peroxidase (GPX)] in black porgy exposed to thermal (20 °C→30 °C) and hypoosmotic (35 psu→10 psu and 0 psu) stresses. The expression and activity of antioxidant enzymes were significantly higher after exposure to 30 °C, 10 psu, and 0 psu. Furthermore, we measured H_2O_2 and lipid peroxidation (LPO) levels. As a result, H_2O_2 and LPO levels were significantly increased after exposure to thermal (20 °C→30 °C) and hypoosmotic stress (35 psu→10 psu and 0 psu) stress. These results indicate that thermal and hypoosmotic stress induces oxidative stress in black porgy. Additionally, we investigated the changes due to thermal and hypoosmotic stress by measuring plasma cortisol and ion (Na^+ and Cl^-) levels. Plasma cortisol levels increased at 30 °C and at 10 psu and then decreased at 0 psu. However, plasma Na^+ and Cl^- levels did not change after exposure to thermal stress (30 °C), and decreased at 10 psu and 0 psu. In conclusion, thermal and hypoosmotic environments increase oxidative stress, thereby these results may be indicators of oxidative stress in black porgy.

1. Introduction

Stress factors in fish can be divided into 2 groups: physical factors, such as salinity, temperature, culture density, temperature, dissolved oxygen, and chemical factors (Beckmann et al., 1990). Physical factors, such as salinity and temperature change, affect growth, reproduction, metabolism, osmoregulation, and immune function, causing negative effects under physiological conditions, such as a disturbance in growth and reproduction (Ackerman et al., 2000). Furthermore, stress induced by changes in salinity has been associated with enhanced reactive oxygen species (ROS) generation, which may seriously affect immune function and lead to oxidative stress (Paital and Chainy, 2010; Shin et al., in press).

ROS, including superoxide (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radicals (OH^-), and singlet oxygen ($^1O_2$), are produced naturally during oxidative metabolism (Roch, 1999). Overproduction of ROS in response to environmental stress can lead to increased lipid peroxidation (LPO) and may affect cell viability by causing membrane damage and enzyme inactivity (Nordberg and Arnér, 2001). Subsequently, cell senescence and apoptosis and oxidation of nucleic acids and proteins may be accelerated. The resultant DNA damage may provoke a variety of physiological disorders such as accelerated aging, reduced disease resistance, and reduced reproductive ability (Kim and Phyllis, 1998; Pandey et al., 2003).

Complex antioxidant defense systems maintain homeostasis and protect aerobic organisms against ROS and the subsequent damage of oxidative stress. Antioxidants may be enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), and glutathione S-transferase (GST), as well as compounds such as metallothionein, quercetin, vitamin C, and vitamin E (α-tocopherol) (McFarland et al., 1999; Jayaraj et al., 2007). Antioxidant defense systems are found in the livers and kidneys of marine organisms (Basha Siraj and Rani Usha, 2003). Both SOD and CAT directly scavenge ROS. SOD removes O_2^- through the process of dismutation to O_2 and H_2O_2 ($2O_2^- + 2H^+ \rightarrow H_2O_2 + O_2$; Kashiwagi et al., 1997). The H_2O_2 produced by Cu/Zn-SOD is sequentially reduced to H_2O and O_2 by CAT (Kashiwagi et al., 1997). CAT is an oxidoreductase that breaks down 2 molecules of H_2O_2 into 2 molecules of H_2O and O_2 ($2H_2O_2 \rightarrow 2H_2O + O_2$), thereby counteracting the toxicity of H_2O_2 (Kashiwagi et al., 1997).

Black porgy (*Acanthopagrus schlegeli*) is an euryhaline teleost that moves from coastal waters to near-shore shallow areas during the transition from larvae to juveniles and lives in coastal waters near land or in estuaries and in 18–21 °C water (Kinoshita and Tanaka, 1990). In summer, water temperature is increased, and then stress and death of the fish occurs than in any other season (Collazos et al., 1995). Therefore, we investigated the expression and activity of antioxidant enzymes and the changes in the H_2O_2, and LPO levels in black porgy to understand the oxidative stress induced by thermal

* Corresponding author. Tel.: +82 51 410 4756; fax: +82 51 404 4750.
E-mail address: choic@hhu.ac.kr (C.Y. Choi).

1095-6433/$ – see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.cbpa.2010.02.013

and hypoosmotic stress. In addition, we analyzed changes in Na^+, Cl^-, and cortisol levels to provide basic data about the physiological responses and the index of stress induced by thermal and hypoosmotic stresses in black porgy.

2. Materials and methods

2.1. Experimental fish and conditions

The study was performed with 1-year-old black porgy (*Acanthopagrus schlegeli*, Sparidae) ($n = 60$, 14.3 ± 0.4 cm, 51.0 ± 6.0 g) reared in three 220-L circulating filter tanks in the laboratory. Before the experiment, water temperature and photoperiod were 20 ± 1 °C and 12L:12D, respectively.

2.2. Temperature changes

Fish were reared in seawater in the two circulating filter tanks (40 L) with automatic temperature regulation systems (JS-WBP-170RP; Johnsam Co., Seoul, Korea) and allowed to acclimatize to the conditions for 24 h. The water temperature was then increased from 20 °C to 30 °C in daily increments of 1 °C. No fish died during the increase in water temperature.

2.3. Salinity change

Transfer of black porgy to a hypoosmotic environment was performed according to the methods of Min et al. (2003). Briefly, underground water was poured into the circulating filter tanks (40-L) and the water kept at 10 psu for 24 h, and then underground water was again added to convert the water in the tanks to 0 psu for 24 h. The water temperature and photoperiod were maintained at 20 ± 1 °C and 12L:12D, respectively. No fish died during the salinity change.

2.4. Sampling

Five fish from each group (35 psu seawater, 10 psu seawater, 0 psu freshwater and 30 °C water temperature) were randomly selected for tissue and blood collection and anesthetized with tricaine methanesulfonate (200 mg/L, MS-222; Sigma, St. Louis, MO, USA); blood was taken from the caudal vasculature using a 3 mL heparinized syringe. After centrifugation (12,000 g, 4 °C, 5 min), the plasma was stored in at −80 °C before analysis, and fish were killed by spinal transection for collection of the liver. Immediately after sample collection, the livers were frozen in liquid nitrogen and stored at −80 °C until total RNA extraction was performed.

2.5. Quantitative PCR (QPCR)

QPCR was conducted to determine the relative expression of Cu/Zn-SOD, CAT and GPX mRNA in the total RNA extracted from the liver. Primers for QPCR were designed with reference to the known sequences of black porgy (GenBank accession no. Cu/Zn-SOD: AJ000249, CAT: GU370345, GPX: GU799605) as follows: Cu/Zn-SOD forward primer (5′-GTT GCC AAG ATA GAC ATC AC-3′), Cu/Zn-SOD reverse primer (5′-TTA GAC TCT CCT CGT TGC-3′), CAT forward primer (5′-GCA ACT ACC AGC GTG ATG-3′), CAT reverse primer (5′-CAG ACA CCT TGA ACT TGG A-3′), GPX forward primer (5′-CAG GAG AAC GGC AAG AAT-3′), GPX reverse primer (5′-TTC CAT TCA CAT CCA CCT T-3′), β-actin forward primer (5′-GCA AGA GAG GTA TCC TGA CC-3′) and β-actin reverse primer (5′-CTC AGC TCG TTG TAG AAG G-3′). PCR amplification was conducted using an iCycler iQ Multicolor Real-Time PCR Detection System (Bio-Rad, Hercules, CA, USA) and iQ™ SYBR Green Supermix (Bio-Rad) according to the manufacturer's instructions. QPCR was performed as follows: denaturation at 95 °C for 5 min, 35 cycles of denaturation at 95 °C for 20 s, annealing at 55 °C for 20 s and extension at 72 °C for 20 s. After the PCR program, QPCR data from three replicate samples were analyzed with analysis software of the system (Bio-Rad) to estimate transcript copy numbers for each sample. The efficiencies of the reactions were determined by performing the QPCR. The efficiencies were found to be as follows: β-actin = 95.3%, Cu/Zn-SOD = 96.7%, CAT = 97.0% and GPX = 95.1%. All data were expressed as change with respect to the corresponding β-actin calculated cycle threshold (ΔCt) levels. The calibrated ΔCt value (ΔΔCt) for each sample and internal control (β-actin) was calculated [$\Delta\Delta Ct = 2^{\wedge} - (\Delta Ct_{sample} - \Delta Ct_{internal\ control})$]. Also, to ensure that the primers amplified a specific product, we performed a melt curve, melting at only one temperature.

2.6. Cu/Zn-SOD, CAT and GPX activity analysis

The tissues were homogenized in ice-cold 0.1 M phosphate-buffered saline (PBS, pH 7.4) containing 1 mM ethylenediaminetetraacetic acid (EDTA). The homogenates were centrifuged at $10{,}000 \times g$ for 15 min at 4 °C; the supernatant was removed and then the remaining sample was used for analysis. Cu/Zn-SOD, CAT and GPX activities were determined using commercial kits supplied by Cayman Chemical (Ann Arbor, MI, USA) and measured the activity according to the manufacture's instruction.

Cu/Zn-SOD activity was assessed by using a tetrazolium salt for detecting superoxide radicals generated by xanthine oxidase and hypoxanthine. One unit of Cu/Zn-SOD is defined as the amount of enzyme needed to exhibit 50% dismutation of the superoxide radical. Absorbance was read at 450 nm. Each assay was performed in duplicate, and enzyme units were recorded as U/mL. For CAT activity, the assay is based on the reaction of the enzyme with methanol in the presence of an optimal concentration of H_2O_2. The formaldehyde produced is measured spectrophotometrically with 4-amino-3-hydrazino-5-mercapto-1,2,4-triazole (Purpald®) as the chromogen (Wheeler et al., 1990). Purpald® specifically forms a bicyclic heterocycle with aldehydes, which upon oxidation changes from colorless to purple. Absorbance was read at 540 nm. Each assay was performed in duplicate, and CAT activity was expressed in nmole/min/mL. GPX activity measured indirectly by a coupled reaction with glutathione reductase. Oxidized glutathione, produced upon reduction of hydroperoxide by GPX, is recycled to its reduced state by glutathione reductase and NADPH. The oxidation of NADPH to $NADP^+$ is accompanied by a decrease in absorbance at 340 nm, and GPX activity expressed in nmol/min/mL.

2.7. H_2O_2 assay

H_2O_2 concentrations were measured using the modified methods of Nouroozzadeh et al. (1994) and a Peroxidetect Kit (Sigma). The 20 µL of black porgy plasma was added per well to flat-bottom 96-well plates. Plates were left at room temperature for 20 min to allow the plasma to settle and adhere to the plate. A working color reagent was prepared by mixing 100 mL distilled water containing 100 mM sorbitol and 125 µM xylenol orange (Sigma) with 1 mL of 25 mM ferrous ammonium sulfate prepared in 2.5 M sulfuric acid (Sigma). Two hundred microliters of this reagent was then added to each well and allowed to incubate at room temperature for 1 h. Absorbance was read at 560 nm and the concentration of H_2O_2 was interpolated from a standard curve. Concentrations are expressed as nM/mL.

2.8. LPO assay

LPO is quantified by measuring malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE), the degradation products of polyunsaturated fatty acids (PUFAs) hydroperoxides (Esterbauer et al., 1991), and is measured according to the manufacture's instructions (Lipid Hydroperoxide Assay Kit, Cayman Chemical). Tissue (1 g) was homogenized in 10 mL of HPLC-grade water. The 500 µL of the

cytosolic fraction of the homogenate were added glass tube. The 500 μL of chloroform, 450 μL of chloroform–methanol, and 50 μL of ferric thiocyanide solution (FTS) reagent 1 and FTS reagent 2 mixtures (Cayman chemical) were added glass tube and mixed. This sample incubated for 5 min at room temperature. Samples (300 μL per well) were added to flat bottom 96 well plates. The absorbance was read at 500 nm using a plate reader. LPO is expressed as nM of MDA and 4-HNE/g protein.

2.9. Plasma parameters analysis

Plasma Na^+ and Cl^- were examined using a biochemistry autoanalyzer (Hitachi, Japan). And plasma cortisol was analyzed by radioimmunoassay (RIA) using a commercial RIA kit (Diagnostic System Laboratories, Webster, TX, USA).

2.10. Statistical analysis

All data were analyzed using the SPSS statistical package (version 10.0; SPSS Inc., USA). One way ANOVA followed by Tukey's *post hoc* was used to compare the differences to control in the data ($P<0.05$).

3. Results

3.1. Expression of antioxidant enzymes induced by thermal and hypoosmotic stress

Cu/Zn-SOD and CAT mRNA expressions significantly increased by about 5-fold, and GPX mRNA significantly increased by about 3.8-fold in the 30 °C group as compared to those in the control group (20 °C) (Fig. 1). Cu/Zn-SOD and CAT mRNA expressions significantly increased by about 17.5-fold and 13-fold, respectively, and GPX mRNA significantly increased by about 7.3-fold at 10 psu, and then decreased at 0 psu (Fig. 2).

3.2. Activity of antioxidant enzymes induced by thermal and hypoosmotic stress

Cu/Zn-SOD and CAT activities significantly increased by about 2-fold, and GPX activity significantly increased by about 1.3-fold in the 30 °C group as compared to those in the 20 °C group (Fig. 1). Cu/Zn-SOD and CAT activities significantly increased by about 2.5-fold, and GPX activity significantly increased by about 1.2-fold at 10 psu, and then decreased at 0 psu (Fig. 2).

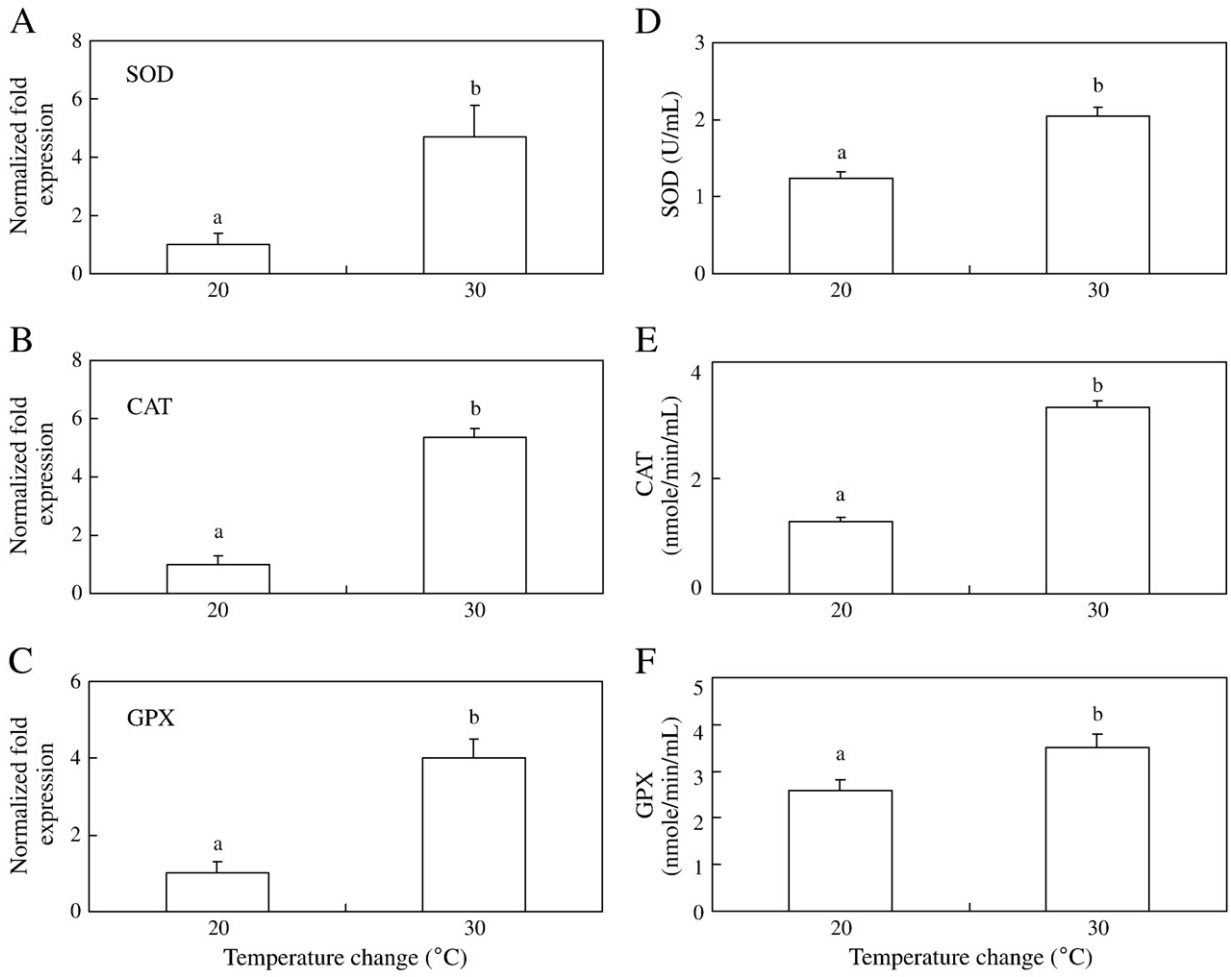

Fig. 1. Expression of Cu/Zn-superoxide dismutase (Cu/Zn-SOD) (A), catalase (CAT) (B), glutathione peroxidase (GPX) (C) mRNA as measured by quantitative real-time polymerase chain reaction (PCR), and activity of Cu/Zn-SOD (D), CAT (E) and GPX (F) in black porgy exposed to thermal stress (30 °C). Total RNA (2.5 μg) was reverse-transcribed and amplified by using gene-specific primers. The results are expressed as normalized fold expression with respect to β-actin in the same sample. Different letters indicate significant differences ($P<0.05$). All values are mean ± standard deviation (SD) ($n=5$).

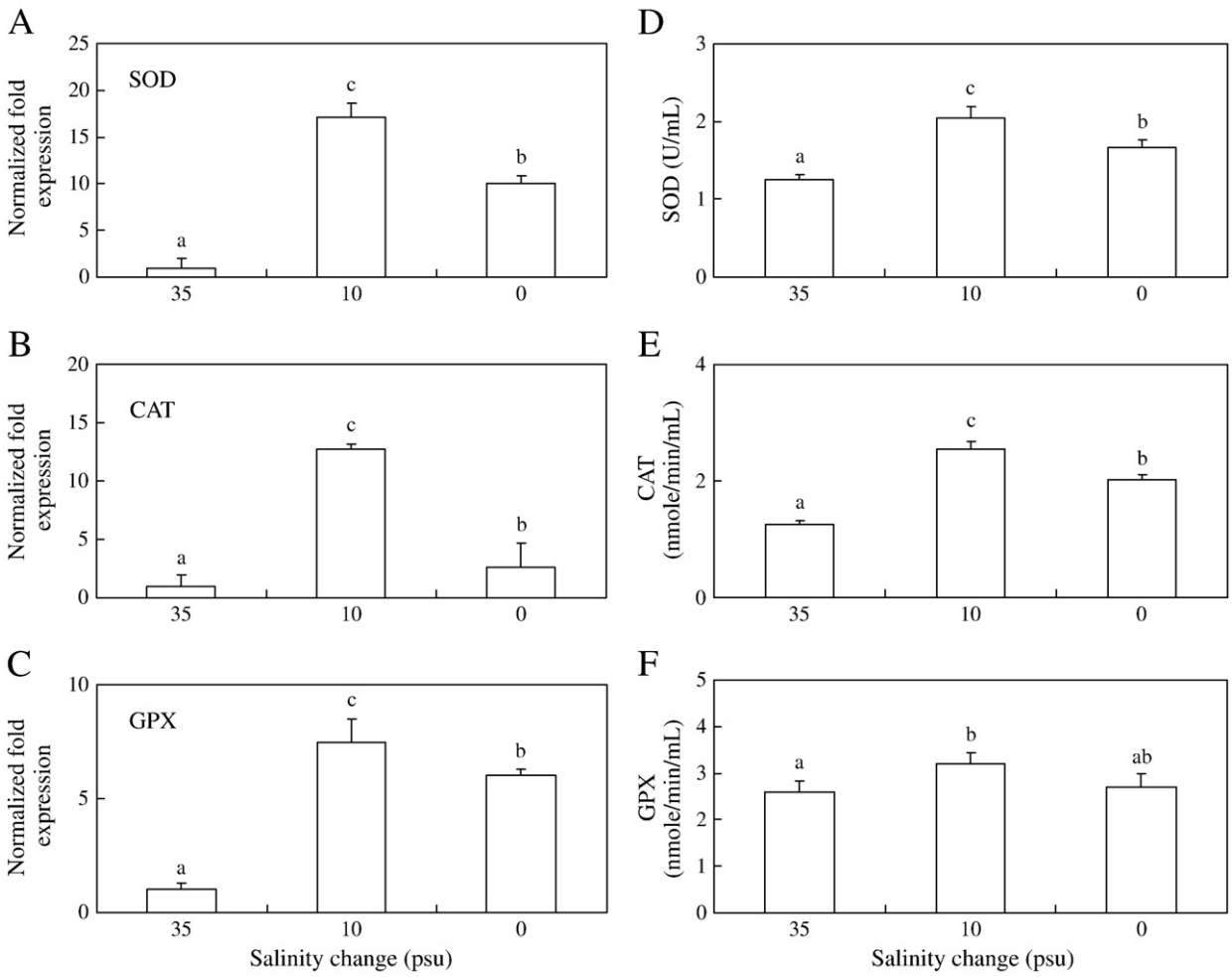

Fig. 2. Expression of Cu/Zn-superoxide dismutase (Cu/Zn-SOD) (A), catalase (CAT) (B), glutathione peroxidase (GPX) (C) mRNA as measured by quantitative real-time polymerase chain reaction (PCR), and activity of Cu/Zn-SOD (D), CAT (E) and GPX (F) in black porgy exposed to hypoosmotic stress (10 psu and 0 psu). Total RNA (2.5 μg) was reverse-transcribed and amplified by using gene-specific primers. The results are expressed as normalized fold expression with respect to β-actin in the same sample. Different letters indicate significant differences ($P<0.05$). All values are mean ± standard deviation (SD) ($n=5$).

3.3. Effects of thermal and hypoosmotic stresses on H_2O_2 levels

At a temperature of 20 °C (35 psu), the plasma H_2O_2 level was 66.2 ± 7.1 nmol peroxide/mL in the control group. It significantly increased to 191.4 ± 8.7 nmol peroxide/mL at 30 °C (Fig. 3). At a salinity level of 10 psu, the H_2O_2 level increased to 210.4 ± 5.8 nmol peroxide/mL. It then decreased to 166.7 ± 11.2 nmol peroxide/mL at 0 psu (Fig. 4).

3.4. Effects of thermal and hypoosmotic stresses on LPO levels

At 35 psu (20 °C), the level of LPO (expressed as malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE) compounds) was 57.3 ± 6.8 μM, and it significantly increased to 131.5 ± 6.2 μM at 30 °C (Fig. 3). The level of LPO increased to 114.1 ± 12.3 μM at 10 psu and 119.2 ± 5.7 μM at 0 psu (Fig. 4).

3.5. Effects of thermal and hypoosmotic stresses on plasma parameters

The plasma cortisol level was 9.0 ng/mL in the control group, but it significantly increased to 47.5 ng/mL at 30 °C (Table 1). It increased to 18.7 ng/mL at 10 psu and 32.5 ng/mL at 0 psu (Table 2). The plasma Na^+ and Cl^- levels were 179.5 mEq/L and 153 mEq/L, respectively, in the control group, but there were no significant differences in these parameters between the 20 °C and 30 °C groups (Table 1). The plasma Na^+ and Cl^- levels decreased to 171 mEq/L and 138.3 mEq/L, respectively, at 0 psu (Table 2).

4. Discussion

This study was performed to understand the oxidative stress induced in the body by rapid environmental changes, such as the transfer of black porgy to high water temperatures or hypoosmotic environments. We measured the expression and activity of antioxidant enzymes (Cu/Zn-SOD, CAT and GPX) as well as an index of oxidative stress (H_2O_2 and LPO) to investigate the oxidative stress induced by high water temperatures and hypoosmotic environments. In addition, we investigated the physiological changes by measuring plasma Na^+, Cl^-, and cortisol levels.

It is known that rapid changes in water temperature and salinity induce oxidative stress by increasing the amount ROS in fish (Shin et al., in press). In the present study, black porgy reared at 20 °C were transferred to high water temperature (30 °C), and we then measured the mRNA expression and activity of Cu/Zn-SOD, CAT and GPX in the liver. We found high levels of these parameters at 30 °C (Fig. 1). Our results are in agreement with those of a previous report that found increased GPX activity in goldfish exposed to high temperatures (3 °C → 23 °C) (Bagnyukova et al., 2007). Moreover, Cu/Zn-SOD activity increased when goldfish were exposed to 35 °C water temperature

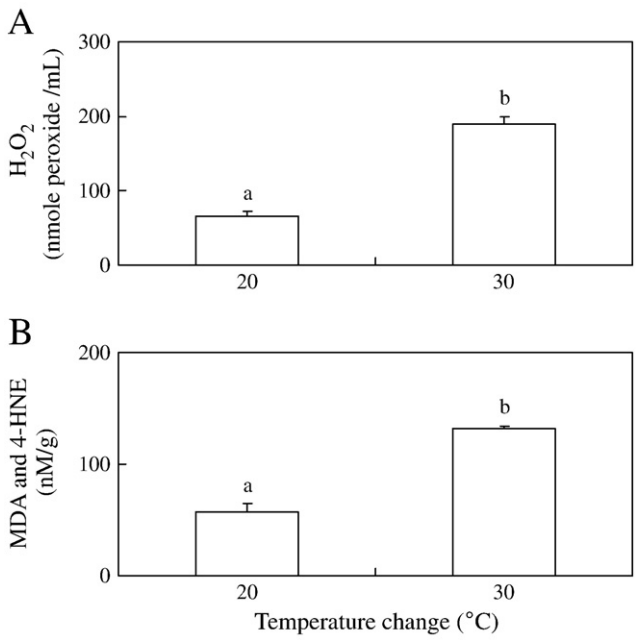

Fig. 3. Plasma H_2O_2 levels (A) and malondialdehyde (MDA) and 4-HNE levels (B) in black porgy exposed to thermal stress (30 °C). Different letters indicate significant differences ($P<0.05$). All values are mean ± standard deviation (SD) ($n=5$).

(Lushchak and Bagnyukova, 2006a, b) and in mice exposed to 38 °C temperature (Djordjević et al., 2004), indicating a possible key role of SOD in protection against ROS produced at high temperatures (Lushchak and Bagnyukova, 2006a, b). Thermal stress accelerates the oxidation of polyamine in cells to generate ROS (Harari et al., 1989); thus, oxidative stress induced by ROS may be closely connected with the antioxidant response (Abele et al., 1998; Liu et al., 2007). Furthermore, to examine the ROS levels induced by thermal stress, we investigated H_2O_2 levels in plasma. We found that the plasma H_2O_2 concentration was significantly increased at 30 °C (Fig. 3). Thus, oxidative stress was induced by thermal stress, and this result supports the increase in the

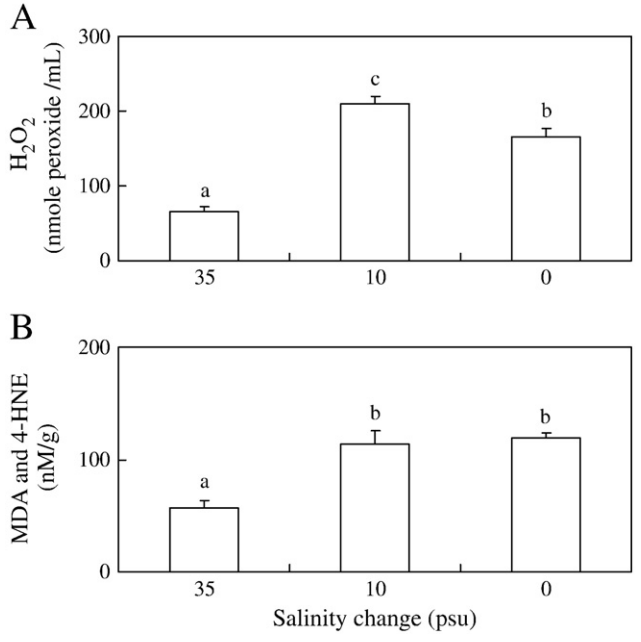

Fig. 4. Plasma H_2O_2 levels (A) and malondialdehyde (MDA) and 4-HNE levels (B) in black porgy exposed to hypoosmotic stress (10 psu and 0 psu). Different letters indicate significant difference ($P<0.05$). All values are mean ± standard deviation (SD) ($n=5$).

Table 1
Plasma Na^+, Cl^- and cortisol levels during temperature changes in black porgy.

Ambient	Na^+ (mEq/L)	Cl^- (mEq/L)	Cortisol (ng/mL)
20 °C	179.5 ± 2.5	153.0 ± 2.3	9.0 ± 3.0[a]
30 °C	180.3 ± 1.1	159.3 ± 1.0	47.5 ± 2.3[b]

Different letters indicate significant differences ($P<0.05$). All values are mean ± standard deviation (SD) ($n=5$).

expression and activity of antioxidant enzymes (Cu/Zn-SOD, CAT and GPX) caused by thermal stress in the present study. Moreover, when the fish are under oxidative stress induced by exposure to high water temperatures, the LPO levels increase as do the expression and activity of antioxidant enzymes (Abele et al., 1998; Bagnyukova et al., 2007). In this study, when black porgy were exposed to 30 °C temperature, the LPO level increased (Fig. 3); this result is in agreement with that of a previous study on goldfish, wherein the products of LPO, lipid peroxides (LOOH) and thiobarbituric acid-reactive substances (TBARS), increased due to oxidative stress induced by thermal stress (35 °C) and MDA levels increased in thornfish exposed to 36 °C temperature (Chien and Hwang, 2001).

It is known that the aquatic organisms were under oxidative stress induced by salinity changes as well as high water temperatures (Halliwell and Gutteridge, 1989; Liu et al., 2007; Shin et al., in press). Black porgy reared at 35 psu were gradually transferred to 10 psu or 0 psu, and we investigated the expression and activity of antioxidant enzymes (Cu/Zn-SOD, CAT and GPX). The levels of these enzymes tended to increase at 10 psu and decrease at 0 psu (Fig. 2). These results are similar to that of a previous study that found that the expression of GPX mRNA in olive flounder increased by exposure to a hypoosmotic environment (Choi et al., 2008). The activity of Cu/Zn-SOD and CAT increased in mud crab by exposure to a hypoosmotic environment (10 psu) (Paital and Chainy, 2010), and the expression and activity of Cu/Zn-SOD and CAT in ark shell increased by exposure to a hypoosmotic environment (An and Choi, 2010).

The mRNA expression and activity of Cu/Zn-SOD elevated when black porgy was exposed to high temperature and hypoosmotic environments. High temperature and hypoosmotic stresses might have produced a large amount of ROS in liver which could have induced Cu/Zn-SOD to scavenge superoxide radicals. Produced H_2O_2 after removed superoxide radicals were scavenged by CAT and GPX. In high temperature and hypoosmotic environments, the mRNA expression and activity of CAT and GPX were similar with each other, so produced H_2O_2 was removed by CAT and GPX as same function (Chae et al., 1999). These results concur with those of previous study reported that CAT and GPX activities significantly increased as same patterns (Li et al., 2010).

Moreover, to examine the levels of ROS induced by hypoosmotic stress, we measured the plasma H_2O_2 concentration and found that the plasma H_2O_2 concentration significantly increased at 10 psu (Fig. 4). On the basis of these results, we suggest that oxidative stress in black porgy increased in a hypoosmotic environment (10 psu), but it decreased at 0 psu leading to a decrease in H_2O_2 concentration. However, LPO was maintained at high levels at 0 psu; thus, we found that LPO was constantly induced, despite the decrease in oxidative stress at 0 psu (Fig. 4).

Table 2
Plasma Na^+, Cl^-, and cortisol levels during salinity changes in black porgy.

Ambient	Na^+ (mEq/L)	Cl^- (mEq/L)	Cortisol (ng/mL)
35 psu	179.5 ± 2.5[a]	153.0 ± 2.3[a]	9.0 ± 3.0[a]
10 psu	176.8 ± 1.9[a]	145.5 ± 1.0[b]	18.7 ± 2.2[b]
0 psu	171.0 ± 2.9[b]	138.3 ± 3.9[c]	32.5 ± 4.7[c]

Different letters indicate significant differences ($P<0.05$). All values are mean ± standard deviation (SD) ($n=5$).

Although black porgy is the euryhaline teleost (Kimura and Tanaka, 1991), it was under oxidative stress induced by rapid salinity changes. We suggest that the expression and activity of antioxidant enzymes decreased because black porgy had already adapted to 0 psu freshwater, and this concurred with those of previous study reported that black porgy adapted in 0 psu (Chang et al., 2007). Thus, we hypothesize that in black porgy the expression and activity of the antioxidant enzymes increased as a defense system against oxidative stress (high water temperatures and hypo-salinity environment).

Furthermore, when the fish are under stress induced by rapid environmental changes, the plasma cortisol levels increase (Barton and Iwama, 1991). In the present study, when black porgy that were reared at 20 °C and then transferred to 30 °C, plasma cortisol levels were high at 30 °C (Table 1). When Atlantic cod (Pérez-Casanova et al., 2008) and Chinook salmon (Barton and Schreck, 1987) were exposed to high water temperatures, the plasma cortisol levels increased; thus, we believe that high water temperatures induce stress in fish.

We detected that the plasma cortisol levels significantly increased at 10 psu. The levels increased at 0 psu after transfer from 35 psu to 10 psu and 0 psu (Table 2). We believe that rapid salinity changes increased the plasma cortisol levels. This was also noted in a previous study in which cortisol levels significantly increased when black porgy was transferred from seawater to freshwater (Chang et al., 2002) and when tilapia was transferred from freshwater to seawater (Chang and Hur, 1999). However, although black porgy are euryhaline fish with a strong salinity tolerance (Kimura and Tanaka, 1991), we hypothesize that they are under stress at hypoosmotic environment (10 and 0 psu) because of a rapid salinity change, so the cortisol levels increased. In fish, it is well known that cortisol is involved in hypoosmoregulation and ion regulation in teleots (Mommsen et al., 1999; Mancera et al., 2002). Also, there are many studies that cortisol plays a role in ion uptake in teleosts (Mancera et al., 2002). Therefore, in the present study, it seems that cortisol was increased for regulation of ion levels. In addition, plasma Na^+ and Cl^- levels did not change significantly when black porgy were transferred from 20 °C to a high water temperature environment (30 °C) (Table 1); thus, we believe that the stress induced by a high water temperature environment did not affect osmolality.

On the other hand, the ion levels in fish changed due to rapid salinity changes. In this study, when black porgy were gradually transferred from 35 psu to 10 psu and 0 psu, the plasma Na^+ and Cl^- levels tended to decrease during the experimental period (Table 2). This is in agreement with the results of a previous study in which the levels of Na^+ and Cl^- in plasma decreased in flatfish (Sampaio and Bianchini, 2002). Thus, we believe that the levels of Na^+ and Cl^- in plasma decreased after transfer to hypoosmotic environment because the salinity content of the water was lower than that of their bodies; ions were released outside and water flowed inward to maintain the body's osmolality (Mancera et al., 1993, McCormick, 1995).

In conclusion, oxidative stress was induced in black porgy by exposure to thermal and hypoosmotic environments. Therefore we concluded that these stress factors increased expression and activity of Cu/Zn-SOD, CAT and GPX to remove ROS, and these factors lead to LPO. So we suggested that black porgy have the antioxidant system against these stress factors. Black porgy adapted to the hypoosmotic environment (0 psu) because euryhaline teleost fish have a strong salinity tolerance, but we concluded that black porgy were stressed by the rapid salinity change. Moreover, we suggest that markers of oxidative stress increased in a high water temperature environment. The fact that black porgy were stressed by exposure to the high water temperature and hypoosmotic environment is an obvious fact. In this study, we investigated the levels of ROS that can be expected to be induced due to environmental changes, and cortisol as a biomarker of stress. Furthermore, these results, along with antioxidant enzymes (expression of mRNA and activity) and LPO generated by various environmental stresses can provide basic data about oxidative stress and antioxidant mechanisms.

Acknowledgements

This research was supported by the MKE (The Ministry of Knowledge Economy), Korea, under the ITRC (Information Technology Research Center) support program supervised by the NIPA (National IT Industry Promotion Agency (NIPA-2009-C1090-0903-0007).

References

Abele, D., Burlando, B., Viarengo, A., Pörtner, H.O., 1998. Exposure to elevated temperatures and hydrogen peroxide elicits oxidative stress and antioxidant response in the Antarctic intertidal limpet *Nacella concinna*. Comp. Biochem. Physiol. B 120, 425–435.

Ackerman, P.A., Forsyth, R.B., Mazur, C.F., Iwama, G.K., 2000. Stress hormones and the cellular stress response in salmonids. Fish Physiol. Biochem. 23, 327–336.

An, M.I., Choi, C.Y., 2010. Activity of antioxidant enzymes and physiological responses in ark shell, *Scapharca broughtonii*, exposed to thermal and osmotic stress: Effects on hemolymph and biochemical parameters. Comp. Biochem. Physiol. B 155, 34–42.

Bagnyukova, T.V., Lushchak, O.V., Storey, K.B., Lushchak, V.I., 2007. Oxidative stress and antioxidant defense responses by goldfish tissues to acute change of temperature from 3 to 23 °C. J. Therm. Biol. 32, 227–234.

Barton, B.A., Schreck, C.B., 1987. Influence of acclimation temperature on interrenal and carbohydrate stress responses in juvenile Chinook salmon (*Oncorhynchus tshawytsc*). Aquaculture 62, 299–310.

Barton, B.A., Iwama, G.K., 1991. Physiological changes in fish from stress in aquaculture with emphasis on the response and effects of corticosteroids. Annu. Rev. Fish Dis. 1, 3–26.

Basha Siraj, P., Rani Usha, A., 2003. Cadmium-induced antioxidant defense mechanism in freshwater teleost *Oreochromis mossambicus* (Tilapia). Ecotoxical. Environ. Saf. 56, 218–221.

Beckmann, R.P., Mizzen, L.E., Welch, W.J., 1990. Interaction of HSP70 with newly synthesized proteins: implications for protein folding and assembly. Science 248, 850–854.

Chae, H.Z., Kim, H.J., Kang, S.W., Ghee, S.G., 1999. Characterization of three isoforms of mammalian peroxiredoxin that reduce peroxides in the presence of thioredoxin. Diabetes Res. Clin. Pract. 45, 101–112.

Chang, Y.J., Hur, J.W., 1999. Physiological responses of grey mullet (*Mugil cephalus*) and Nile tilapia (*Oreochromis niloticus*) by rapid changes in salinity of rearing water. J. Korean Fish. Soc. 32, 310–316.

Chang, Y.J., Min, B.H., Chang, H.J., Hur, J.W., 2002. Comparison of blood physiology in black porgy (*Acanthopagrus schlegeli*) cultured in converted freshwater from seawater and seawater from freshwater. J. Korean Fish. Soc. 35, 595–600.

Chang, Y.J., Min, B.H., Choi, C.Y., 2007. Black porgy (*Acanthopagrus schlegeli*) prolactin cDNA sequence: mRNA expression and blood physiological responses during freshwater acclimation. Comp. Biochem. Physiol. B 147, 122–128.

Chien, L.T., Hwang, D.F., 2001. Effects of thermal stress and vitamin C on lipid peroxidation and fatty acid composition in the liver of thornfish *Terapon jarbua*. Comp. Biochem. Physiol. B 128, 91–97.

Choi, C.Y., An, K.W., An, M.I., 2008. Molecular characterization and mRNA expression of glutathione peroxidase and glutathione S-transferase during osmotic stress in olive flounder (*Paralichthys olivaceus*). Comp. Biochem. Physiol. A 149, 330–337.

Collazos, M.E., Barriga, C., Ortega, E., 1995. Effect of high summer temperatures upon granulocyte phagocytic function of the tench (*Tinca tinca*, L.). Comp. Immun. Microbiol. Infect. Dis. 18, 115–121.

Djordjević, J., Cvijić, G., Vučković, T., Davidović, V., 2004. Effect of heat and cold exposure on the rat brain monoamine oxidase and antioxidative enzyme activities. J. Therm. Biol. 29, 861–864.

Esterbauer, H., Schaur, R.J., Zoliner, H., 1991. Chemistry and biochemistry of 4-hydroxynonenal, malonaldehyde and related aldehydes. Free Radic. Biol. Med. 11, 81–128.

Halliwell, B., Gutteridge, J.M.C., 1989. Free radicals in Biology and Medicine. Clarendon Press, Oxford, pp. 96–98.

Harari, P.M., Fuller, D.J., Gerner, E.W., 1989. Heat shock stimulates polyamine oxidation by two distinct mechanisms in mammalian cell cultures. Int. J. Radiat. Oncol. Biol. Phys. 16, 451–457.

Jayaraj, R., Deb, U., Bhaskar, A.S.B., Prasad, G.B.K.S., Lakshmana Rao, P.V., 2007. Hepatoprotective efficacy of certain flavonoids against microcystin induces toxicity in mice. Environ. Toxicol. 22, 472–479.

Kashiwagi, A., Kashiwagi, K., Takase, M., Hanada, H., Nakamura, M., 1997. Comparison of catalase in diploid and haploid *Rana rugosa* using heat and chemical inactivation techniques. Comp. Biochem. Physiol. B 118, 499–503.

Kim, M.O., Phyllis, E.B., 1998. Oxidative stress in critical care: is antioxidant supplementation beneficial? J. Am. Diet. Assoc. 98, 1001–1008.

Kimura, R., Tanaka, M., 1991. Prolactin production during larval and early juvenile periods of euryhaline marine fish, black sea bream *Acanthopagrus schlegeli*. Nippon suisan gakkaishi/bull. Jpn. Soc. Sci. Fish. 57, 1833–1837.

Kinoshita, I., Tanaka, M., 1990. Differentiated spatial distribution of larvae and juveniles of the two sparids, red and black sea bream, in Shijiki Bay. Nippon Suisan Gakkaishi 56, 1807–1813.

Li, Z.H., Zlabek, V., Velisek, J., Grabic, R., Machova, J., Randak, T., 2010. Modulation of antioxidant defence system in brain of rainbow trout (*Oncorhynchus mykiss*) after chronic carbamazepine treatment. Comp. Biochem. Physiol. C 151, 137–141.

Liu, Y., Wang, W.N., Wang, A.L., Wang, J.M., Sun, R.Y., 2007. Effects of dietary vitamin E supplementation on antioxidant enzyme activities in *Litopenaeus vannamei* (Boone, 1931) exposed to acute salinity changes. Aquaculture 265, 351–358.

Lushchak, V.I., Bagnyukova, T.V., 2006a. Temperature increase results in oxidative stress in goldfish tissues: 1. Indices of oxidative stress. Comp. Biochem. Physiol. C 143, 30–35.

Lushchak, V.I., Bagnyukova, T.V., 2006b. Temperature increase results in oxidative stress in goldfish tissues: 2. Antioxidant and associated enzymes. Comp. Biochem. Physiol. C 143, 36–41.

Mancera, J.M., Perez-Figares, J.M., Fernandez-Llebrez, P., 1993. Osmoregulatory responses to abrupt salinity changes in the euryhaline gilthead sea bream (*Sparus aurata* L.). Comp. Biochem. Physiol. A 106, 245–250.

Mancera, J.M., Carrión, R.L., del Río, M.P.M., 2002. Osmoregulatory action of PRL, GH, and cortisol in the giltead seabream (*Sparus aurata* L.). Gen. Comp. Endocrinol. 129, 95–103.

McCormick, S.D., 1995. Hormonal control of gill Na^+, K^+-ATPase and chloride cell function. In: Wood, C.M., Shuttleworth, T.J. (Eds.), Cellular and molecular approaches to fish ionic regulation, vol. 14. Academic Press, San Diego, pp. 285–315.

McFarland, V.A., Inouye, L.S., Lutz, C.H., Jarvis, A.S., Clarke, J.U., McCant, D.D., 1999. Biomarkers of oxidative stress and genotoxicity in livers of field-collected brown bullhead, *Ameiurus nebulosus*. Arch. Environ. Contam. Toxicol. 37, 236–241.

Min, B.H., Kim, B.K., Hur, J.W., Bang, I.C., Byun, S.K., Choi, C.Y., Chang, Y.J., 2003. Physiological responses during freshwater acclimation of seawater-cultured black porgy (*Acanthopagrus schlegeli*). Korean J. Ichthyol. 15, 224–231.

Mommsen, T.P., Vijayan, M., Moon, T., 1999. Cortisol in teleosts: dynamics, mechanisms of action, and metabolic regulation. Rev. Fish Biol. Fisheries 9, 211–268.

Nordberg, J., Arnér, E.S.J., 2001. Reactive oxygen species, antioxidants, and the mammalian thioredoxin system. Free Radic. Biol. Med. 31, 1287–1312.

Nouroozzadeh, J., Tajaddinisarmadi, J., Wolff, S.P., 1994. Measurement of plasma hydroperoxide concentrations by ferrous oxidation-xylenol orange assay in conjunction with triphenylphosphine. Anal. Biochem. 200, 403–409.

Paital, B., Chainy, G.B.N., 2010. Antioxidant defenses and oxidative stress parameters in tissues of mud crab (*Scylla serrata*) with reference to changing salinity. Comp. Biochem. Physiol. C 151, 142–151.

Pandey, S., Parvez, S., Sayeed, I., Haques, R., Bin-Hafeez, B., Raisuddin, S., 2003. Biomarkers of oxidative stress: a comparative study of river Yamuna fish Wallago attu (Bl & Schn.). Sci. Total Environ. 309, 105–115.

Pérez-Casanova, J.C., Rise, M.L., Dixon, B., Afonso, L.O.B., Hall, J.R., Johnson, S.C., Gamperl, A.K., 2008. The immune and stress responses of Atlantic cod to long-term increases in water temperature. Fish Shell. Immunol. 24, 600–609.

Roch, P., 1999. Defense mechanisms and disease prevention in farmed marine invertebrate. Aquaculture 172, 125–145.

Sampaio, L.A., Bianchini, A., 2002. Salinity effects on osmoregulation and growth of the euryhaline flounder *Paralichthys orbignyanus*. J. Exp. Mar. Biol. Ecol. 269, 187–196.

Shin, H.S., Yoo, J.H., Min, T.S., Lee, K.Y., Choi, C.Y., in press. The effects of quercetin on physiological characteristics and oxidative stress resistance in olive flounder, *Paralichthys olivaceus*. Asian-Aust. J. Anim. Sci.

Wheeler, C.R., Salzman, J.A., Elsayed, N.M., 1990. Automated assays for superoxide dismutase, catalase, glutathione peroxidase, and glutathione reductase activity. Anal. Biochem. 184, 193–199.