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Black porgy (*Acanthopagrus schlegeli*) prolactin cDNA sequence: mRNA expression and blood physiological responses during freshwater acclimation

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Abstract

To investigate the consequences of freshwater (FW) transfer, we studied the prolactin (PRL) cDNA sequence and its mRNA expression, and physiological responses in the black porgy (*Acanthopagrus schlegeli*). We cloned and characterized cDNA encoding its PRL from the pituitary gland. Black porgy PRL cDNA consists of 1492 bp and encodes a protein of 212 amino acids including 24 signal peptides. Reverse transcription-PCR showed the PRL mRNA expression in the pituitary gland. Expression of pituitary gland PRL mRNA was significantly higher during FW acclimation. Furthermore, we studied the stress responses and osmoregulatory abilities of black porgy in changing salinities. Plasma cortisol, glucose, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) significantly increased in the fish immediately after transfer to FW. We also identified significant changes in the fish in terms of plasma ions (Na^+, Cl^-, Ca^{2+}) and osmolality during the acclimation period. These results suggests that PRL plays an important role in hormonal regulation in osmoregulatory organs, thereby improving the hyperosmoregulatory ability of black porgy in freshwater.

Keywords: Black porgy; *Acanthopagrus schlegeli*; Freshwater acclimation; Osmoregulation; Prolactin; Stress responses

1. Introduction

Black porgy (*Acanthopagrus schlegeli*) are euryhaline teleosts, and move from coastal waters to near shore shallow areas during their transition from larvae to juveniles and live in coastal waters near land or in estuaries (Kinoshita and Tanaka, 1990; Tanaka et al., 1991). Being euryhaline, black porgies have excellent osmoregulatory abilities (Kitajima and Tsukashima, 1983).

Prolactin (PRL), a peptide hormone secreted from the pituitary gland, plays an important role in ion regulation by facilitating retention/uptake of Na^+ in FW (Loretz and Bern, 1982; Hirano, 1986; McCormick, 2001). Plasma PRL levels were found to decrease in many salmonid fishes when they were transferred from FW to SW (Prunet and Boeuf, 1989; Young et al., 1989; Yada et al., 1991), and tilapia showed increased levels of plasma PRL when transferred from SW to FW (Yada et al., 1994; Shepherd et al., 1999). Thus, PRL activity levels are higher in FW than in SW (Nagahama et al., 1975; Nishioka et al., 1988).

In aquaculture, salinity changes cause a variety of physiological stress responses including plasma hormones, energy metabolism and electrolyte equilibrium reactions (Barton and Iwama, 1991; Iwama et al., 1994). Corticosteroid increases in the blood due to salt stress have been reported in several species (Brown et al., 2001; Frisch and Anderson, 2005). The primary stress responses trigger secondary responses such as glucose increases in blood related to increased energy requirements (Carmichael et al., 1984; Barton and Iwama, 1991), electrolyte homeostasis changes in blood and tissues (Carmichael et al., 1984; McDonald and Milligan, 1997), and increases in aspartate aminotransferase (AST) and alanine aminotransferase (ALT) (De Smet and Blust, 2001; Almeida et al., 2002).

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Although a great deal of study has been conducted on the FW culture of the black porgy, little research has focused on the physiological changes in response to FW acclimation. The objective of this study was to examine the role of PRL in the black porgy during FW acclimation by analyzing the base sequence after cloning the full length of PRL cDNA of black porgy and then measuring the level of pituitary mRNA based on this result. We also examined the stress response and osmoregulatory ability of the black porgy with regard to FW acclimation by measuring the levels of plasma cortisol, glucose, AST, ALT, ions, and osmolality.

2. Materials and methods

2.1. Experimental animals and FW acclimation

Black porgy (average length 14.3±0.4 cm, weight 51.0± 6.0 g) were collected from a commercial fish farm and reared in 220-l circulation filter tanks in the laboratory. No feed was supplied during the experimental period. FW acclimation of the black porgy was performed according to the methods of Min et al. (2003). Briefly, underground water was poured into the tank and the fish were kept at 10‰ SW for 24 h, when underground water was again added to convert the water in the tanks to completely FW. Water temperature and light were maintained at 20 °C and a 12L/12D cycle, respectively.

2.2. Sampling procedure

Six fish from each treatment [SW, 10‰ SW (24 h), FW (24 h), FW (48 h), and FW (72 h)] were randomly selected for blood and tissue sampling. Fish were anesthetized with a 200 mg/l solution of tricaine methanesulfonate (MS-222) prior to blood collection. Blood was collected from the caudal vasculature using a 3-ml syringe coated with heparin. Plasma samples were separated by centrifugation and stored at −80 °C until analysis. Fish were killed by spinal transection for the collection of the pituitary sample. Immediately after collection, the pituitary sample was refrigerated in liquid nitrogen and stored at −80 °C until analysis by RT-PCR.

2.3. Isolation of PRL cDNA

The degenerate primers were designed from highly conserved regions of PRL from the sea bream (*Sparus aurata*: GenBank accession no. AF060541) and flounder (*Paralichthys olivaceus*: GenBank accession no. AF047616). The sequence of the forward primer [5′-TGT TGT (AG)CA TGG TGG CAG C-3′] was based on nucleotides 103–121; the sequence of the reverse primer [5′-AGC G(AC)A GGA C (AG)T TCA GGA A-3′] was based on nucleotides 645–663. Total RNA was extracted using a TRIzol kit (Gibco/BRL, Grand Island, NY, USA). Reverse transcription and PCR amplification were conducted using the AccuPower RT/PCR PreMix (Bioneer, Daejeon, Korea) according to the manufacturer's instructions. A single PCR product of the expected size (561 bp) was obtained. The amplified DNA was extracted using a plasmid purification kit (NucleoGen, Seoul, Korea) and sequencing was performed by COSMO (Seoul, Korea).

2.4. Rapid amplification of cDNA 3′- and 5′-ends (RACE)

For RACE reactions, total RNA was isolated from the pituitary as described above. With 2 μg of total RNA as a template, 5′-RACE-ready cDNA and 3′-RACE-ready cDNA were generated using protocols and reagents provided in a CapFishing full-length cDNA premix kit (Seegene, Seoul, Korea). Gene specific primers were selected from the 561 bp PCR product obtained by RT-PCR. For 5′-RACE, the 50 μl PCR reaction mixture was as follows: 5 μl of 5′-RACE-ready cDNA, 1 μl of 10 mmol l^{-1} 5′-target primer, 1 μl of 10 mmol l^{-1} 5′ RACE gene specific primer (5′-GSP: 5′-TGT CAT TGG GTG TCT GTA GAG AGG AGG TAT-3′), and 25 μl of SeeAmp Taq Plus master mix (Seegene). PCR was carried out for 35 cycles as follows: denaturation at 94 °C for 45 s, annealing at 62 °C for 45 s and extension at 72 °C for 90 s, followed by 1 cycle of 5 min at 72 °C for extension. The PCR product was amplified, cloned into a pGEM-T Easy Vector (Promega, Madison, WI, USA), and sequenced. For 3′-RACE, the 50 μl PCR reaction mixture contained 3′-RACE-ready cDNA, 3′-gene specific primer (3′-GSP: 5′-TCA CTG CCC TAC AGA GGC TCC AAT GAC A-3′), 3′ target primer, and SeeAmp Taq Plus master mix, as described above. PCR conditions were same as previously described. The PCR product was amplified, cloned into a pGEM-T Easy Vector, and sequenced. DNA and deduced amino acid sequence data were analyzed using GENETYX-WIN (Software Development Co., Tokyo, Japan).

2.5. Reverse transcription-polymerase chain reaction (RT-PCR)

Total RNA was extracted from the pituitary of black porgy. PRL specific primers for RT-PCR were designed as follows: forward primer: [5′-TGT CAT TGG GTG TCT GTA GAG AGG AGG TAT-3′, reverse primer: 5′-TCA CTG CCC TAC AGA GGC TCC AAT GAC A-3′]. One microgram of total RNA was reverse transcribed for 30 min at 50 °C using oligo(dT) primer and SuperScript™ reverse transcriptase (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. Equal amounts of RNAs (as determined spectrophotometrically) from the various tissues were used for reverse transcription. RT-PCR was performed using an AccuPower RT/PCR PreMix (Bioneer). As a control, black porgy β-actin mRNA was also amplified for each RT reaction product. The β-actin primers were 5′-TCG AGC GTA TTG TGA CC-3′ for the forward primer and 5′-ACG GAA CCT CTC ATT GCC GA-3′ for the reverse primer. Quantification of PCR amplified fragment was carried out by high resolution scanner and the band densities were estimated by a computer program (KBT Co., Korea). In each case the loading was controlled by amplification of β-actin. The densitometric process from ethidium bromide-stained gel was optimized for linearity as described previously (Kermouni et al., 1998).

2.6. Plasma parameters analysis

Plasma cortisol was analyzed by radioimmunoassay (RIA) using an RIA kit (Diagnostic System Laboratories, Webster, TX, USA). Plasma glucose, AST, ALT, Na^+, Ca^{2+} and Cl^- were analyzed using the Biochemistry Auto analyzer (model 7180; Hitachi, Tokyo, Japan). Plasma osmolality was examined with a Vapor Pressure Osmometer (Vapro 5520; Wescor Co., Logan, UT, USA).

2.7. Statistical analysis

All data were analyzed using the SPSS (version 10.0; SPSS Inc., Chicago, IL, USA) statistical package. One way ANOVA followed by a *post hoc* multiple comparison test (Tukey's test) was used to compare differences in the data.

3. Results

3.1. Identification of PRL cDNA

A PCR-based cloning strategy (RT-PCR followed by 3′- and 5′-RACE) was used to clone a cDNA encoding a putative PRL from the black porgy pituitary gland. The 1492 bp cDNA includes an open reading frame (ORF) of 639 bp that began with the first ATG codon at position 60 bp and ended with a TAA stop codon at position 698 bp (GenBank accession no. AY929158). The amino acid sequence of black porgy PRL is compared to those deduced from cDNAs of other teleost species in Fig. 1. Amino acid identities of PRL with other fish species are as follows: 92% with sea bream (GenBank accession no. AF060541), 79.7% with sea bass (GenBank accession no. X78723), 73.6% with flounder (GenBank accession no. AF047616), and 69% with pufferfish (GenBank accession no. AB164301). Black porgy PRL contained four cysteine residues, the positions of which were well conserved among teleost PRLs.

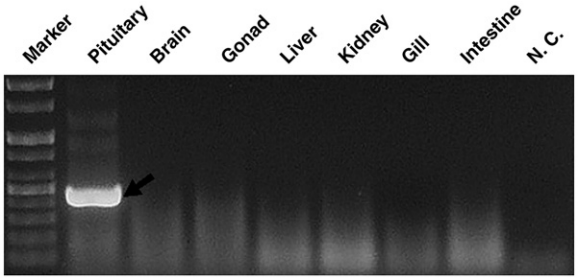

Fig. 2. Tissue distribution of prolactin (PRL) in black porgy (*Acanthopagrus schlegeli*) by RT-PCR. N.C. represents negative control. Arrow indicates expected size of PRL (561 bp).

3.2. Tissue distribution analysis of PRL mRNA

Result of RT-PCR for PRL is shown in Fig. 2. A single band of the expected size (561 bp) was obtained in the pituitary, but no band was detected in the other tissues examined.

3.3. PRL mRNA expression in black porgy pituitary

The RT-PCR technique showed the expression of PRL mRNA in the pituitary of black porgy during the experiment, but expression varied among the different treatments. In the 10‰ SW treatment, expression was significantly higher compared to that in SW, whereas the peak PRL mRNA expression was found in fish in the FW treatment (72 h; Fig. 3). The significantly lowest mRNA expression of PRL was observed in fish in the SW treatment.

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bpPRL     1:MARRETNGSKLFITVLCMVAACGA-VPISDLLDRASQRSDTLHSLSTTLTQDLNNHIPPV  59
ofPRL     1:MTHRR-TKLFMMAAVVSYVMTSCGAVPINDLLDRASQRSDQLHSLSTTLSQELDSHFPPI  59
pfPRL     1:MAHRKPGDILLLVTVLCMVATARGTVSTSDLLDRVSEHSDMIHSLSTILSQDLDSQLPPV  60
bsPRL     1:MAQRKTNGSKLFMMVLYMVAACSA-IPISDLLDRASQRSDTLHSLSTTLTQDLDSHFPPM  59
sbPRL     1:MAHRETNGSKLFITVLCMVAACSA-VPINDLLDRASQRSDMLHSLSTTLTKDLSNHVPPV  59

bpPRL    60:GWMMMPRPSTCHTSSLQTPNDKEQALQLSESDLLSLARSLLQAWQDPLVVLSNSANSLVH 119
ofPRL    60:GRVIMPRPSMCHTSALQTPNDKTQALQVSESELLSLARSLLQAWADPLSALSSSAFSLPH 119
pfPRL    61:GRMLLPRPSMCHTSSLQTPMDKEQALQISKSDLLSLARSLLHAWADPLLFLSTSAMTLPQ 120
bsPRL    60:GRVITPRPSMCHTSSLHTPIDKEQALQVSEADLLSLVRSLLQAWRDPLVILSTSANTLPH 119
sbPRL    60:GWTMMPRPPLCHTSSLQTPNDKEQALQLSESDLMSLARSLLQAWQDPLVDLSNSANSLLH 119
                    *

bpPRL   120:PSQSSISNKIRELQEHSKSLGDGLDILSGKMGPEAQAISSLPYRGSNDIGEDSISKLTNF 179
ofPRL   120:PAQSSIFNKVREMQEHSKNLGDGLDILSGKMGEAAQALSSLPFR-GNDVGQDRISKLINF 178
pfPRL   121:LAQSSVSNKIQELKQHSQTLGDGLNILSDRMGQAAQAISSLPYSGGNDLGQDKISKLINL 180
bsPRL   120:PAQNSISTKVQELLEHTKSLGDGLDILSGKFGPAAQSISSLPYRGGNDISQDRISRLTNF 179
sbPRL   120:PSQSSISNKIRELQEHSKSLGDGLDILSGKMGPAAQAISSLPYRGSNDIGEDNISKLTNF 179

bpPRL   120:HFLLSCFRRDSHKIDSFLNVLRCRAAKLQPEMC                          212
ofPRL   120:HFLLSCFRRDSHKIDSFLKVLRCRAANTQPEMC                          211
pfPRL   121:HFLLSCFRRDSHKIDSFLKVLRCRMANMVPEMC                          213
bsPRL   120:HFLMSCFRRDSHKIDSFLKVLRCRAAKLQPEMC                          312
sbPRL   120:HFLLSCFRRDSHKIDSFLKVLRCRAAKVQPEMC                          212
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Fig. 1. Comparison of the amino acid sequences of black porgy (*Acanthopagrus schlegeli*) PRL, olive flounder (*Paralichthys olivaceus*) PRL, pufferfish (*Takifugu rubripes*) PRL, sea bass (*Dicentrarchus labrax*) PRL, and sea bream (*Sparus aurata*) PRL, optimally aligned to match identical residues, indicated by the shaded box. Sequences were taken from the GenBank/EMBL/DDBJ sequence databases. The PRL sequences used for alignment are black porgy PRL (bpPRL, AY929158), olive flounder PRL (ofPRL, AF047616), pufferfish PRL (pfPRL, AB164301), sea bass PRL (bsPRL, X78723), and sea bream PRL (sbPRL, AF060541). The signal peptide of black porgy PRL is boxed. An asterisk indicates four cysteine residues.

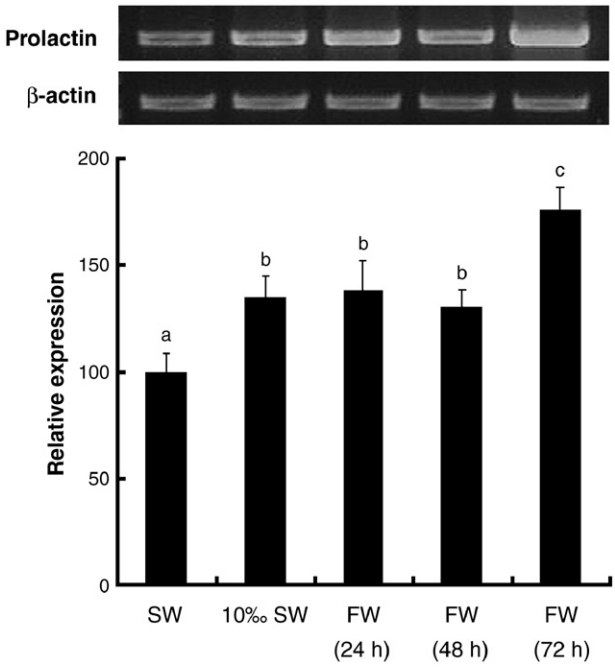

Fig. 3. Expression of PRL mRNA during FW acclimation in black porgy (*Acanthopagrus schlegeli*) reared in SW. One microgram of total RNA prepared from the pituitary gland was reverse transcribed and amplified using black porgy PRL-specific primer. The pituitary PRL of black porgy was analyzed by RT-PCR. The expression of β-actin mRNA was evaluated in each RT reaction product as a control. The expression level of each tissue was normalized with respect to the β-actin signal and expressed as relative expression level. Each value represents the mean±SE (n=6). Same letters indicate no significant difference (P>0.05).

3.4. Plasma parameters

Plasma cortisol levels were 5.3±1.3 ng/ml in SW, reaching their highest level of 32.0±6.4 ng/ml in the FW (24 h) treatment. Levels then started to decrease to a similar level as found in the 10‰ SW (24 h) treatment. Plasma glucose levels showed similar changing patterns as cortisol, with no significant differences between SW and FW (72 h; Fig. 4). AST and ALT also showed similar patterns of change as plasma cortisol and glucose (Fig. 5). AST and ALT were at their peak when fish were transferred to FW for 24 h. Fish that had been in FW for 24 h showed significantly higher AST than the fish from SW, FW (48 h), and FW (72 h) treatments. No differences were observed in plasma Na^+ between SW and 10‰ SW with levels of 179.5±2.5 mEq/l and 176.8±1.9 mEq/l, respectively. However, Na^+ decreased significantly in FW, exhibiting the lowest value (171.0±2.9 mEq/l) at 24 h, and then showing a gradual increase. Plasma Cl^- was significantly higher in SW, decreasing to its lowest value of 138.3±3.9 mEq/l in FW (24 h), then again increasing. Plasma Ca^{2+} showed no significant difference between SW and FW (48 h) with levels of 2.0±0.3 to 2.6±0.4 mEq/l. A significantly higher plasma Ca^{2+} was observed in fish in FW (72 h). Plasma osmolality started to decrease from 10‰ SW and showed the lowest levels in FW (24 h), thereafter recovering partially. This pattern of change was similar to that of the plasma Na^+ and Cl^- (Table 1).

4. Discussion

In spite of its multifunctional ability in different classes of vertebrates, PRL is well established as a FW-adapting hormone for teleost species. In search of PRL function in FW-acclimated black porgy, we first cloned cDNA encoding putative PRL from the pituitary. The cloning and sequencing of black porgy PRL cDNA allows for comparison of this sequence with that of other teleosts. After sequencing, we found that the cDNA sequence contained a total of 1492 bp including an open reading frame (ORF) of 639 bp that began with the ATG initiation codon (at position 60 bp) and ended with the TAA stop codon (at position 698 bp). When we compared this sequence to other published sequences we found similarity, indicating that the cDNA chosen was PRL cDNA. PRLs are synthesized from prohormones, which have signal peptides of 23 to 24 amino acids in fish. We confirmed that black porgy PRL ORF encoded a protein of 212 amino acid residues (aa), composed of a putative signal peptide of 24 amino acid and a mature protein of 188 aa. Black porgy PRL is composed of 188 aa and contains four cysteine residues, presumably forming two disulfide bonds of black porgy PRL are formed by $cysteine_{70–186}$ and $cysteine_{203–212}$ in the mature protein, in accordance with other teleost PRLs. Moreover, four cysteine residues are highly conserved in the mature protein accordance with other teleost PRLs. Putative signal peptide of 24 aa contains two cysteine residues. Although mammalian PRLs consist of three disulfide bonds placed in the N-terminal, middle, and carboxyl-terminal regions of the protein, teleost PRLs lack the N-terminal disulfide bond due to the absence of 12 to 14 amino acids at the N-terminus (Rand-Weaver et al., 1993). According to Manzon (2002), the fact that teleosts have no disulfide bridge in the N-terminus may be related to the

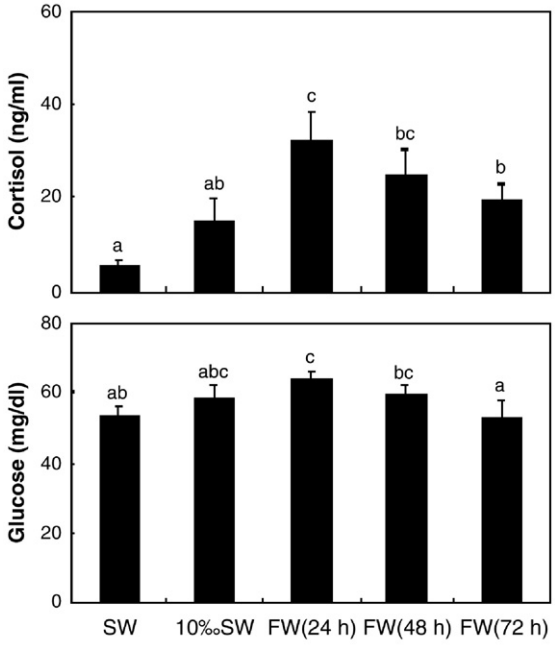

Fig. 4. Levels of plasma cortisol and glucose during FW acclimation of black porgy (*Acanthopagrus schlegeli*) reared in SW. Each value represents the mean±SE (n=6). Same letters indicate no significant difference (P>0.05).

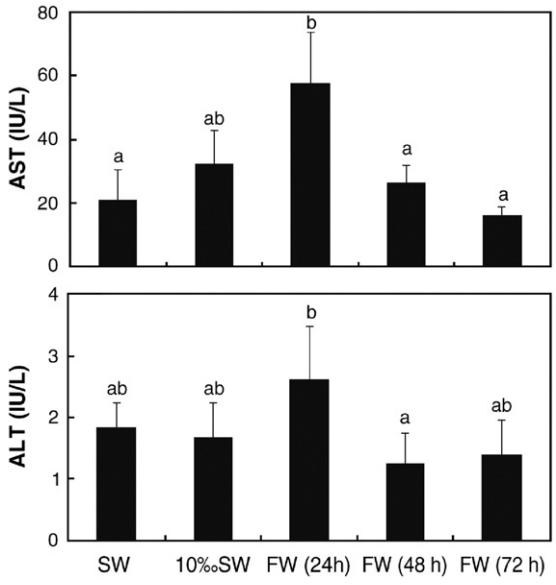

Fig. 5. Levels of AST and ALT during FW acclimation of black porgy (*Acanthopagrus schlegeli*) reared in SW. Each value represents the mean±SE (n=6). Same letters indicate no significant difference (P>0.05).

central role of PRL as an osmoregulatory hormone, which is supported by the finding that osmoregulation in teleosts was significantly improved by ovine PRL in which the disulfide bridge was disrupted in the N-terminus (Doneen et al., 1979).

Two types of PRL cDNAs have been characterized in chum salmon (*Oncorhynchus keta*) (Yasuda et al., 1986), eel (*Anguilla japonica*) (Suzuki et al., 1991), tilapia (*Oreochromis mossambicus*) (Yamaguchi et al., 1988) and Nile tilapia (*Oreochromis niloticus*) (Rentier-Delrue et al., 1989). In tilapia, which have two PRLs, PRL_{188} (188 amino acids) and PRL_{177} (177 amino acids), their homology is as low as 69% compared to other fish (Yamaguchi et al., 1988). While PRL_{188} is mainly related to the regulation of plasma Na^+ and Cl^-, PRL_{177} is related to the decrease of water permeation (Auperin et al., 1994).

In our present study, we examined the expression of pituitary PRL mRNA through RT-PCR. The expression of black porgy PRL was detected in the pituitary, while no band was observed in the other tissues examined (Fig. 2). This agrees with a previous report (Lee et al., 2006) that the expression of pufferfish PRL was only detected in the pituitary. In seabream, however, PRL is expressed not only in the pituitary but also in the liver, intestine, ovary and testis (Santos et al., 1999), although it is not clear whether the PRL expressions in those extra-pituitary organs are of some physiological significance.

Expression of PRL mRNA was found to increase in the pituitary as salinity decreased, indicating that an increase of plasma PRL in black porgy with reduced salinity. The mRNA expression pattern in black porgy that were transferred to FW closely parallels the mRNA expression pattern in tilapia (Yada et al., 1994; Sakamoto et al., 1997; Riley et al., 2003), Atlantic salmon (Martin et al., 1999), and pufferfish (Lee et al., 2006). This increase in the mRNA expression of PRL supports the hypothesis that PRL is essential for FW acclimation in teleosts through the inhibition of Na^+/K^+-ATPase activity as described by Sakamoto et al. (1997). Although in this study, pituitary PRL mRNA expression was significantly lower in SW than in 10‰ SW, PRL has been reported to play an important role in osmoregulation in marine teleosts such as pufferfish (Lee et al., 2006). As noted previously, PRL receptor can be expressed not only in osmoregulatory organs such as the gills, intestines, and kidneys but also in the gonads (Santos et al., 2001). This means that PRL shows functional diversity, with additional uses other than in osmoregulation.

Plasma cortisol is potentially a valuable hormonal index of stress. This study demonstrated that black porgy are sensitive to salinity changes as indicated by the significant increases in plasma cortisol concentrations. In the present study, FW transfer caused an immediate increase in plasma cortisol that reached its peak within 24 h and then started to decrease. This result agrees with that of Chang et al. (2002). Such stress responses with regard to cortisol change can be explained by two points: abrupt salinity changes cause more stress to fish than gradual changes, and after a salinity change, cortisol returns to its original level as the fish becomes acclimated to the new environment. This phenomenon can be observed in several fish species (*Anguilla anguilla*: Leloup-Hatey, 1974; *Oncorhynchus kisutch*: Redding and Schreck, 1983: *Fundulus heteroclitus*: Jacob and Taylor, 1983; *Sparus aurata*: Mancera et al., 1993; *Plectropomus leopardus*: Frisch and Anderson, 2005).

Generally, glucose is increased by stress. The increase of plasma glucose is caused by cortisol, which enhances the gluconeogenic capacity of phosphoenolpyruvate carboxykinase (PEPCK), a gluconeogenic enzyme in the liver (Hanson and Reshef, 1997). In this study, plasma glucose increased following a similar pattern to that of cortisol, suggesting that cortisol mediates the gluconeogenesis process.

AST and ALT are amino transfer enzymes and a general index of liver function in vertebrates. In fish, they are used to evaluate the stress responses caused by temperature change, low oxygen, pH, ammonia, or heavy metals (Pan et al., 2003). In this study as shown in Fig. 5, the AST and ALT levels in black porgy showed similar patterns to those of cortisol and glucose during FW acclimation, suggesting that the liver cells of black porgy are destroyed, and liver function is degraded due to salt stress.

In SW, fish release ions and absorb water, whereas in FW, fish absorb ions and release water in the course of osmoregulation according to the osmotic gradient; thereby osmolality remains stable. In our study, when black porgy were transferred from SW to FW, plasma Na^+, Cl^-, Ca^{2+} and osmolality decreased to their lowest level within 24 h in FW, and then

Table 1
Levels of Na^+, Cl^-, Ca^{2+} and osmolality during FW acclimation in black porgy (*Acanthopagrus schlegeli*) reared in SW

Ambient	Na^+ (mEq/l)	Cl^- (mEq/l)	Ca^{2+} (mEq/l)	Osmolality (mOsm/kg)
SW	179.5 ± 2.5^{a}	153.0 ± 2.3^{a}	2.6 ± 0.4^{ab}	350.0 ± 3.5^{a}
10‰ SW	176.8 ± 1.9^{a}	145.5 ± 1.0^{b}	2.1 ± 0.6^{a}	341.0 ± 5.8^{b}
FW (24h)	171.0 ± 2.9^{c}	138.3 ± 3.9^{c}	2.0 ± 0.3^{a}	329.8 ± 8.4^{c}
FW (48h)	173.8 ± 2.2^{b}	149.2 ± 1.9^{b}	2.1 ± 0.6^{a}	337.0 ± 3.5^{b}
FW (72h)	174.0 ± 1.1^{b}	148.0 ± 4.3^{b}	3.2 ± 0.4^{b}	334.3 ± 2.6^{bc}

Each value represents the mean±SE (n=6). Same letters indicate no significant difference (P>0.05).

started to recover gradually as ions were regained by the fish. This result is consistent with the findings of several studies (e.g., Mancera et al., 1993; Min et al., 2005). Therefore, it is clear that black porgy, like other euryhaline fish, have excellent osmoregulatory abilities that can be explained through the actions of PRL, a central mediator for FW acclimation (Hirano, 1986; McCormick, 1995). Generally, cortisol rather than PRL is regarded as the SW acclimation hormone (Madsen and Bern, 1992; Seidelin and Madsen, 1997). Indeed, in hypoosmotic media, treatment with cortisol was found to increase the plasma ions (Na^+, Cl^-, Ca^{2+}) and osmolality in rainbow trout, tilapia and catfish (Flik and Perry, 1989; Dang et al., 2000; Eckert et al., 2001). Therefore, this result showed that cortisol is related to hyperosmoregulatory action, and the highest value of cortisol in this study could reflect the minimum levels of osmoregulation.

In this study, physiological changes that are related to stress responses and osmoregulation due to FW acclimation. In our expectation, it can be separated into three periods: a confusion period (up to 24 h in FW), an unstable period of physiological response due to salinity change; an acclimation period (48–72 h in FW), a period of acclimating to the new environment; and a recovery period (72 h to several days in FW), when cortisol hormone and ions have recovered completely to their original levels.

Acknowledgments

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