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Cloning and expression of aquaporin 1 and arginine vasotocin receptor mRNA from the black porgy, *Acanthopagrus schlegeli*: effect of freshwater acclimation

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Abstract We cloned complementary DNA (cDNA) encoding aquaporin 1 (AQP1) and arginine vasotocin receptor (AVT-R) from gill and kidney tissue of the black porgy (*Acanthopagrus schlegeli*), respectively. Black porgy AQP1 cDNA consists of 786 base pairs (bp) and encodes a protein of 261 amino acids, and AVT-R partial cDNA consists of 606 bp. To investigate the osmoregulatory abilities of black porgy in different salinities (35‰ seawater, SW, 10‰ SW, freshwater, FW), we examined the expression of AQP1 and AVT-R mRNA in osmoregulatory organs using the reverse transcription polymerase chain reaction (RT-PCR). AQP1 mRNA levels increased in the gill and intestine during FW acclimation, and the mRNA expression in the kidney was greatest in 10‰ SW and then decreased in FW. On the other hand, AVT-R mRNA was expressed in the gill only in 10‰ SW, while it increased in the kidney in 10‰ SW and then decreased in FW. Thus, the expression of these mRNAs increased in hypoosmotic environments. These results suggest that AQP1 and AVT-R genes play important roles in hormonal regulation in osmoregulatory organs, thereby improving the hyperosmoregulatory ability of black porgy in hypoosmotic environments.

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Introduction

In teleost fish, osmoregulation during changes in salinity is associated with movement of ions, such as Na^+ and Cl^-, and water molecules within gills, kidneys and intestines (Evans 1993; Bentley 2002). In SW fish, the external osmotic pressure is higher than the internal pressure; therefore, the fish take in a large quantity of SW, absorb water through the intestines to replace water loss caused by osmotic stress and discharge Na^+ and Cl^- ions through the gills. SW fish also absorb Na^+ and Cl^- ions through the kidneys and discharge them to the outside environment. It is known that hormones and proteins, such as cortisol, prolactin (PRL), growth hormone (GH), Na^+/K^+-ATPase, arginine vasotocin (AVT) and aquaporins (AQPs), take part in osmoregulation (Pickford and Phillips 1959; Geering 1990; Madsen and Bern 1992; Warne and Balment 1995).

The AQPs is a group of membrane proteins forming water transfer channels that serve an important role in maintaining the water balance in the osmoregulatory organs that control homeostasis of the body fluids (Agre et al. 1993; Borgnia et al. 1999; Ma and Verkman 1999; Matsuzaki et al. 2002). Studies of the role of AQPs in water movement have

been carried out in euryhaline teleosts adapted to FW and SW (Borgnia et al. 1999; Lignot et al. 2002; Aoki et al. 2003; Martinez et al. 2005). So far, 13 types of AQPs cloning have been administered to a variety of organisms, from bacteria to mammals (Chrispeels and Agre 1994; Connolly et al. 1998; Ishibashi et al. 2000; King et al. 2000; Verkman 2002), and the AQPs are divided into three subunits on the basis of the genomic structures and homologues of amino acids. The three subunits are the aquaporins group, which selectively moves water (AQPs 0, 1, 2, 4, 5, 6 and 8; Ishibashi et al. 2000), the aquaglyceroporins group, which is in charge of water, glycerol and urea movement (AQPs 3, 7, 9 and 10; Echevarria et al. 1996; Ishibashi et al. 1997, 1998; Yang and Verkman 1997; Hatakeyama et al. 2001), and the superaquaporins group, which displays low amino acid homologues and indistinct characteristics (AQPs 11 and 12; Ishibashi et al. 2000). Studies so far report that the AQP0 group (Killifish, *Fundulus heteroclitus*, Virkki et al. 2001), AQP1 group (Japanese eel, *Anguilla japonica*, Aoki et al. 2003) and AQP3 group (European eel, *A. anguilla*, Cutler and Cramb 2002; Osorezan dace, *Tribolodon hakonensis*, Hirata et al. 2003; Mozambique tilapia, *Oreochromis mossambicus*, Watanabe et al. 2005; Japanese eel, *A. japonica*, Tse et al. 2006) belong to the teleost fish. Also, expression of the AQPs mRNA was investigated mainly in the gills, kidneys and intestines. AQP1, in particular, is the protein forming a bidirectional channel for water movement and its expression is known to take place in water-permeable tissues, such as lens, gills, red blood cells, renal proximal tubules of kidneys and capillary endothelium (Preston and Agre 1991; Nielsen et al. 1993; Agre et al. 2002).

In this study, experiments were carried out not only on AQPs, but also on the control mechanism and expression of AVT known to be closely associated with osmoregulation.

AVT is a nonapeptide hormone released by the neurohypophysis of teleost fish and other non-mammals (Acher and Chauvet 1995). It activates various physiological functions, such as maintaining blood pressure (Le Mevel et al. 1993; Conklin et al. 1997; Warne and Balment 1997), antidiuretic functions (Henderson and Wales 1974; Amer and Brown 1995) and osmoregulation (Warne and Balment 1995). In mammals, this hormone is similar in function to arginine vasopressin (AVP) (Acher 1996). Changes in the osmotic pressure of a fish's body lead to changes in the concentration of AVT in the plasma (Perrot et al. 1991; Balment et al. 1993; Pierson et al. 1995; Kulczykowska 2001; Warne et al. 2005) and pituitary gland (Perrot et al. 1991; Harding et al. 1997), suggesting that AVT serves an osmoregulatory function. In addition, while mammals have three types of AVP receptors (V_1, V_{1b}, V_2), teleost fish have only one type (V_1). This receptor has been cloned in white suckers (*Catostomus commersonii*) and flounder (*Platichthys flesus*) (Mahlmann et al. 1994; Warne 2001). Although AVT-R performs an important role in AVT activation, its other functions in teleost fish are unclear (Warne 2001).

Black porgy are euryhaline teleosts, which move from coastal waters to nearshore 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 porgy have excellent osmoregulatory abilities (Kitajima and Tsukashima 1983). Studies on the osmoregulation of cultured fish due to salinity changes have been performed on tilapia and several marine species, such as olive flounder, black porgy and European eel (Chang and Hur 1999; Martinez et al. 2005; Cho et al. 2006; Chang et al. 2007).

Although a great many studies have been conducted on the FW culture of the black porgy, little research has focused on the changes of gene expression in response to FW acclimation. Therefore, we investigated the expression patterns of AQP1 and AVT-R mRNA in the osmoregulatory organs of black porgy.

Materials and methods

Experimental fish and FW acclimation

Black porgy (*A. schlegeli*: average length 14.3 ± 0.4 cm, weight 51.0 ± 6.0 g, $n = 50$) were collected from the culture cages of the Marine Science Technology Center (Pukyong National University) and reared in circulation filter SW tanks (220 l) in the laboratory. FW acclimation of the black porgy was performed according to the methods of Min et al. (2003). Briefly, ground water was poured into the

tanks, and the fish were kept at 10‰ SW for 24 h, then underground water was again added to convert the water in the tanks to completely FW. The water temperature and light period were maintained at 20°C and a 12L:12D cycle, and no food was given during the experimental period.

Sampling procedure

Four fish from each salinity (SW, 10‰ SW, FW) were randomly selected for tissue sampling and then anesthetized with tricaine methanesulfonate (MS-222, 200 mg l^{-1}) and killed by spinal transaction for the collection of the tissues (pituitary, brain, liver, testis, gill, kidney and intestine). Immediately after collection, the tissues were frozen in liquid nitrogen and stored at −80°C until total RNA extraction was performed.

Identification of AQP1 cDNA

Mixed primers for AQP1 were designed using highly conserved regions of gilthead seabream (GenBank accession no. AAV34610), European sea bass (GenBank accession no. ABI95464) and Japanese eel (GenBank accession no. BAC82110): bpAQP1 forward primer (5′-AAC CTT CCA GCT GGT GCT GT-3′) and bpAQP1 reverse primer (5′-TCC GTT AAC GTC GTA GTC AC-3′). Total RNA was extracted from the kidney using a TRIzol kit (Gibco/BRL, Grand Island, NY). Reverse transcription (RT) was conducted using M-MLV reverse transcriptase (Bioneer, Seoul, Korea), and polymerase chain reaction (PCR) amplification was performed using a BS Taq Master Mix (Biosesang, Sungnam, Korea) according to the manufacturer's instructions. A single PCR product was purified. The plasmid DNA was extracted using a LaboPass Plasmid DNA Purification Kit (Cosmo, Seoul, Korea), and sequencing was analyzed using an ABI DNA Sequencer (Applied Biosystems, Foster City, CA).

Identification of AVT-R cDNA

Mixed primers for AVT-R were designed using highly conserved regions of European flounder (GenBank accession no. AAF00506), white sucker (GenBank accession no. CAA53958): bpAVT-R forward primer (5′-GCV TCC ACC TAY ATG ATG GTG-3′) and bpAVT-R reverse primer (5′-GTT RCA GCA GCT GTT GAG ACT-3′). Total RNA was extracted from the kidney using the same methods as for AQP1. PCR amplification was conducted using a BS Taq Master Mix (Biosesang, Korea) according to the manufacturer's instructions. Amplified PCR products were processed by electrophoresis in a 1% agarose gel containing ethidium bromide (0.5 μg μl^{-1}). The transformation was conducted using the same methods as for AQP1.

Rapid amplification of AQP1 cDNA 3′ and 5′ ends (3′ and 5′ RACE)

For the PCR reaction, total RNA was extracted from the gill using a TRIzol kit (Gibco/BRL, Grand Island, NY). Using 3 μg of total RNA as a template, 3′ RACE cDNA and 5′ RACE cDNA were synthesized using a CapFishing™ full-length cDNA Premix Kit (Seegene, Korea). First-strand cDNA synthesis was conducted using an oligo (dT) anchor primer (5′-CTG TGA ATG CTG CGA CTA CGA $T(T)_{18}$-3′) and a CapFishing™ adaptor (Seegene, Korea).

AQP1-specific primers were selected from the PCR product obtained by RT-PCR. For the 3′ RACE, the 50 μl of PCR reaction mixture contained 5 μl of 3′ RACE cDNA, 1 μl of 10 mmol l^{-1} 3′ target primer (5′-CTG TGA ATG CTG CGA CTA CGA T-3′), 1 μl of 10 mmol l^{-1} 3′ RACE gene specific primer (5′-TCG CTC CTT TGG TCC GGC TTT GAT CCT-3′) and 25 μl of SeeAmp Taq Plus Master Mix. PCR was carried out for 35 cycles as follows: one cycle of denaturation at 94°C for 5 min, denaturation at 94°C for 40 s, annealing at 62°C for 40 s, and extension at 72°C for 60 s, followed by one cycle of 5 min at 72°C for the final extension.

For 5′ RACE, the 50 μl of PCR reaction mixture contained 5 μl of 5′ RACE cDNA, 1 μl of 10 mmol l^{-1} 5′ target primer (5′-GTC TAC CAG GCA TTC GCT TCA T-3′), 1 μl of 10 mmol l^{-1} 5′ RACE gene specific primer (5′-ACA TGA CCG CCT TGA ACA CGC TGA TCT G-3′) and 25 μl of SeeAmp Taq Plus Master Mix. PCR was carried out for 35 cycles as follows: one cycle of denaturation at 94°C for 5 min, denaturation at 94°C for 40 s, annealing

at 62°C for 40 s and extension at 72°C for 60 s, followed by one final extension cycle of 5 min at 72°C. Amplified PCR products were processed by electrophoresis in a 1% agarose gel containing ethidium bromide (0.5 μg μl^{-1}). The transformation was conducted as the same methods mentioned above.

Semi-quantitative RT-PCR

RT-PCR was conducted to determine the relative expressions of AQP1, AVT-R and β-actin mRNA in black porgy tissues. To optimize the cycle number used for semi-quantitative PCR analysis, the RT reaction (1 μl) from the pituitary gland, gill, kidney, and intestine was used as the template for PCR amplification. AQP1, AVT-R and β-actin-specific primers for RT-PCR were designed from the published sequence: bpAQP1 forward primer (5′-AAC CTT CCA GCT GGT GCT GT-3′) and bpAQP1 reverse primer (5′-TCC GTT AAC GTC GTA GTC AC-3′), bpAVT-R forward primer (5′-CTC GGA TGT TTA CGA CTG CT-3′) and bpAVT-R reverse primer (5′-CTG TTG AGA CTG GCA AGG AG-3′), bpβ-actin forward primer (5′-TCG AGC ACG GTA TTG TGA CC-3′) and bpβ-actin reverse primer (5′-ACG GAA CCT CTC ATT GCC GA-3′). PCR amplification was conducted using a BS Taq Master Mix (Biosesang, Korea) according to the manufacturer's instructions. PCR was carried out as follows: one cycle of denaturation at 94°C for 5 min, denaturation at 94°C for 30 s, annealing at 55°C for 30 s, a total of 33 (AQP1) and/or 40 (AVT-R) cycles for 1 min at 72°C for 30 s, followed by one cycle of 5 min at 72°C for the final extension.

The β-actin mRNA was amplified in each PCR reaction as a loading control. Amplification of β-actin was carried out as follows: one cycle of denaturation at 94°C for 5 min, denaturation at 94°C for 30 s, annealing at 57°C for 30 s, a total of 27 cycles for 1 min at 72°C for 30 s, followed by one cycle of 5 min at 72°C for the final extension. The PCR products from different cycles of amplification were visualized on a UV-transilluminator after electrophoresis on a 1% agarose gel containing ethidium bromide (0.5 μg μl^{-1}), and the signal intensity was quantified with the Gel-Doc System and Gelpro 3.1 software (KBT, Incheon, Korea).

Plasma parameters analysis

Plasma Na^+ 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, 166 UT, USA).

Statistical analysis

The data from each experiment were tested for significant differences using the Statistical Package for the Social Sciences software program (version 10.0; SPSS Inc., Chicago, IL). One-way analysis of variance followed by a post hoc multiple comparison test (Duncan's multiple range test) was used to compare differences in the data at a significance level of $P < 0.05$.

Results

Identification of AQP1 cDNA

RT-PCR was used to clone a fragment of an AQP1 cDNA using total RNA extracted from black porgy gill in FW, where the expression was highest. A single PCR product of the expected size (337 base pairs, bp) was obtained. A PCR-based cloning strategy (RT-PCR followed by 3′ and 5′ RACE) was used to clone a full-length cDNA encoding a putative AQP1 from black porgy gill. AQP1 full-length cDNA contained 786 nucleotides, including an open reading frame (ORF), predicted to encode a protein of 261 amino acids (Fig. 1). The amino acid sequence of black porgy AQP1 is compared to those deduced from the cDNAs of other teleost species, shown in Fig. 1. The amino acid identities of AQP1 with other fish species are as follows: 96% with gilthead seabream (GenBank accession no. AAV34610), 93% with European sea bass (GenBank accession no. ABI95464), 84% with Japanese eel (GenBank accession no. BAC82110) and 75% with zebrafish (GenBank accession no. AAV34608) (Fig. 1).

Tissue distribution of AQP1 mRNA

The expression of AQP1 mRNA during FW acclimation is shown Fig. 2. In the gill and intestine, the

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bpAQP1   1:M-REFKSKDFWRAVLAELVGMTLFIFLSISTAIGNANNTNPDQEVKVSLAFGLAIATLAQ  59
gsAQP1   1:M-REFKSKDFWRAVLAELVGMTLFIFLSISTAIG---STNPDQEVKVSLAFGLAIATLAQ  56
sbAQP1   1:M-REFKSKDFWRAVLAELVGMTLFIYLSISTAIGNPNNSNPDQEVKVSLAFGLAIATLAQ  59
jeAQP1   1:MTKELKSKAFWRAVLAELLGMTLFIFLSIAAAIGNRHNSNPDQEVKVSLAFGLSIATLAQ  60
zfAQP1   1:M-NELKSKAFWRAVLAELLGMTLFIFLSITAAVGNANTQNPDQEIKVALAFGLSIATLAQ  59

bpAQP1  60:SLGHISGAHLNPAVTLGMLASCQISVFKAVMYIVAQMLGSALASGIVYGTRPDTTGGLGL 119
gsAQP1  57:SLGHISGAHLNPAVTLGMLASCQISVFKAVMYIVAQMLGSALASGIVYGTRPSTTDKLGL 116
sbAQP1  60:SLGHISGAHLNPAVTLGMLASCQISVFKAVMYIVAQMLGSALASGIVYGARPSGNLALGL 119
jeAQP1  61:SLGHISGAHLNPAVTLGMLASCQISMLKAVMYVVAQMLGSSVASGIVYGVRPQNNTALGL 120
zfAQP1  60:SLGHISGAHLNPAVTLGLLASCQISLLRAVMYILAQMIGATVASAIVLGV-S-KGDALGL 117

bpAQP1 120:NAL-TGVTPSQGVGIELLATFQLVLCVIAVTDKRRRDVTGSAPLAIGLSVCLGHLAAISY 178
gsAQP1 117:NAL-TGVTPSQGVGIELLATFQLVLCVIAVTDKRRRDVTGSAPLAIGLSVCLGHLAAISY 175
sbAQP1 120:NSL-NNVTPSQGVGIELLATFQLVLCVIAVTDKRRRDVTGSAPLAIGLSVCLGHLAAISY 178
jeAQP1 121:NSL-NEISPSQGVGVEFLATFQLVLCVIATTDKRRRDVTGSAPLAIGLSVALGHLTAISF 179
zfAQP1 118:NQIHTDISAGQGVGIELLATFQLVLCVLATTDKRRRDVSGSAPLAIGLSVCLGHLTAISF 177

bpAQP1 179:TGCGINPARSFGPALILNNFTNHWVYWVGPMCGGVAAALTYDFLLSPKFDDFPERMKVLV 238
gsAQP1 176:TGCGINPARSFGPALILNNFTNHWVYWVGPMCGGVAAALTYDFLLSPKFDDFPERMKVLV 235
sbAQP1 179:TGCGINPARSFGPALILNDFTDHWVYWVGPMCGGVAAALIYDFLLSPKFDDFPERMKVLV 238
jeAQP1 180:TGCGINPARSFGPALILGNFTNHWVYWVGPMCGGVAAALVYDFLLHPKFDDFPERMKVLV 239
zfAQP1 178:TGCGINPARTFGPAMIRLDFANHWVYWVGPMCGGVAAALIYDFLLYPKMDDFPERVRVLV 237

bpAQP1 239:SGPVGDYDVNGGNDATAVEMPSK                                      261
gsAQP1 236:SGPVGDYDVNGGNDATAVEMTSK                                      258
sbAQP1 239:SGPVGDYDVNGGNDATTVEMTSK                                      261
jeAQP1 240:SGPDGDYDVNGPDDVPAVEMSSK                                      262
zfAQP1 238:SGPATDYEVNGTDDPPAVEMSSK                                      260
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Fig. 1 Comparison of the amino acid sequence of black porgy (*Acanthopagrus schlegeli*) AQP1, gilthead seabream (*Sparus aurata*) AQP1, European sea bass (*Dicentrarchus labrax*) AQP1, Japanese eel (*Anguilla japonica*) AQP1 and zebrafish (*Danio rerio*) AQP1 optimally aligned to match identical residues, indicated by the shaded box. The sequences were taken from the GenBank/EMBL/DDBJ sequence databases. The AQP1 sequences used for alignment are black porgy AQP1 (bpAQP1), gilthead seabream AQP1 (gsAQP1, AAV34610), European sea bass AQP1 (sbAQP1, ABI95464), Japanese eel AQP1 (jeAQP1, BAC82110) and zebrafish AQP1 (zbAQP1, AAV34608)

expression of AQP1 mRNA was low in SW and highest in FW (Fig. 2A, C). In contrast, the expression of the mRNA in the kidney was highest in 10‰ SW, and then decreased significantly in FW (Fig. 2B).

RT-PCR using black porgy tissues in FW showed the highest expression level of all the tests performed. A single band of the expected size (337 bp) was observed in all tissues (pituitary, brain, liver, testis, gill, kidney and intestine) (Fig. 4).

Identification of AVT-R partial cDNA

RT-PCR was used to clone a fragment of an AVT-R partial cDNA using total RNA extracted from black porgy kidney in 10‰ SW where the expression was highest. A single PCR product of the expected size (606 bp) was obtained (GenBank accession no. AY929156).

Tissue distribution of AVT-R mRNA

The expression of AVT-R mRNA during FW acclimation is shown Fig. 3. In the gill, AVT-R mRNA was only observed in 10‰ SW; no band was detected in FW (Fig. 3A). In the kidney, the mRNA expression was the highest in 10‰ SW and then decreased in FW (Fig. 3B). However, no AVT-R mRNA was detected in the intestine.

RT-PCR using black porgy tissues in 10‰ SW showed the highest level of expression. A weak single band of the expected size (425 bp) was observed in the pituitary, brain and liver, whereas its expression in the gill and kidney was high. No mRNA was detected in the testis and intestine (Fig. 4).

Plasma parameters

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 the lowest value (171.0 ± 2.9 mEq/l) in FW. Plasma Cl^- was significantly higher in SW, decreasing to its lowest value of 138.3 ± 3.9 mEq/l in FW. Plasma osmolality started to decrease from 10‰ SW and showed the lowest levels in FW (Table 1).

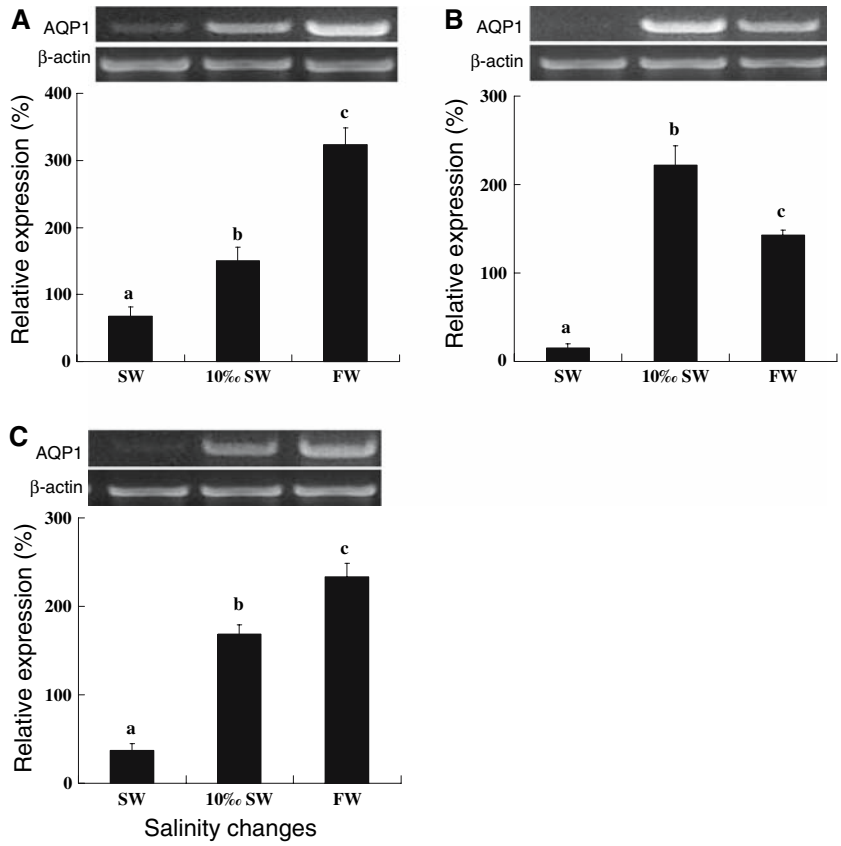

Fig. 2 Expression of AQP1 mRNA in tissues of black porgy. One microgram of total RNA prepared from gill (**A**), kidney (**B**) and intestine (**C**) was reverse transcribed and amplified using black porgy AQP1-specific primer. The tissue distributions of the AQP1 were analyzed by RT-PCR. Values with dissimilar letters are significantly different ($P < 0.05$). Values are means ± SD ($n = 4$)

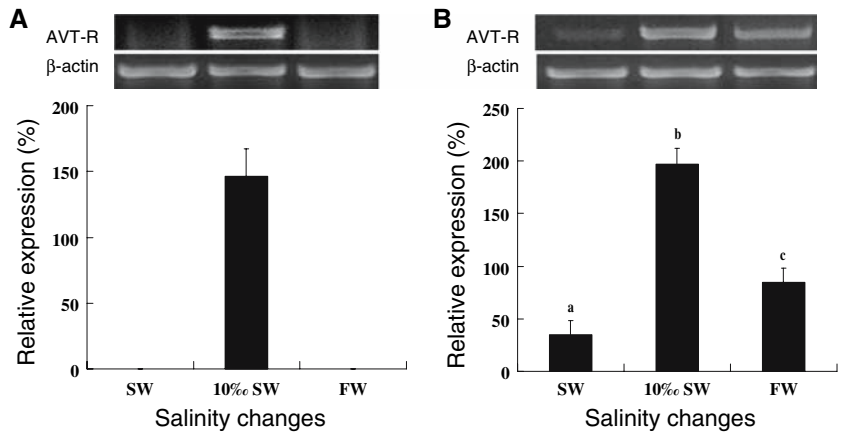

Fig. 3 Expression of AVT-R mRNA in tissues of black porgy. One microgram of total RNA prepared from gill (**A**) and kidney (**B**) was reverse transcribed and amplified using black porgy AVT-R-specific primer. The tissue distributions of black porgy AVT-R were analyzed by RT-PCR. Values with dissimilar letters are significantly different ($P < 0.05$). Values are means ± SD ($n = 4$)

Discussion

In this study, expression of AQP1 and AVT-R genes at the time of cloning and FW adaptation was compared in different tissues of the euryhaline teleost, black porgy. Also, the osmoregulatory capacities of these genes were confirmed by comparing their expression in the tissues of gills, kidneys and intestines, known osmoregulatory organs of aquatic organisms, after FW acclimation. AQP1 from the gills of black porgy contains 261 amino acids in the open reading frame (ORF) between the ATG start codon and the TAA stop codon, and it consists of a total of 786 nucleotides. When compared to those of other teleost fish, the AQP1 amino acids of black porgy displayed a high identity (Fig. 1). Also, AVT-R

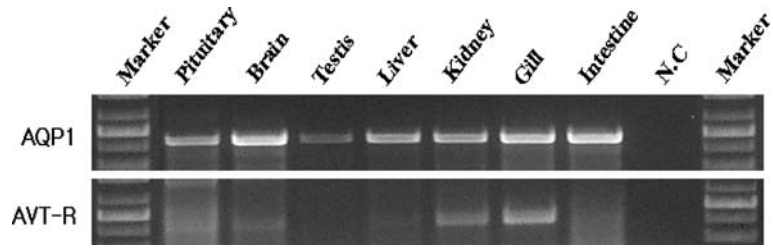

Fig. 4 Expression of AQP1 and AVT-R mRNA in various tissues of black porgy. One microgram of total RNA prepared from pituitary, brain, liver, testis, kidney, gill and intestine was reverse transcribed and amplified using black porgy AQP1 and AVT-R-mixed primer. The tissue distributions of black porgy AQP1 and AVT-R were analyzed by RT-PCR. N.C. represents negative controls. Values with dissimilar letters are significantly different ($P < 0.05$). Values are means ± SD ($n = 4$)

Table 1 Levels of Na^+, Cl^- and osmolality during FW acclimation in black porgy, *Acanthopagrus schlegeli*

Ambient	Na^+ (mEq/l)	Cl^- (mEq/l)	Osmolality (mOsm/kg)
SW	179.5 ± 2.5[a]	153.0 ± 2.3[a]	350.0 ± 3.5[a]
10‰ SW	176.8 ± 1.9[a]	145.5 ± 1.0[b]	341.0 ± 5.8[b]
FW	171.0 ± 2.9[b]	138.3 ± 3.9[c]	329.8 ± 8.4[c]

Values with dissimilar letters are significantly different ($P<0.05$). Values are means ± SD (n=4)

partial cDNA separated from the kidneys of black porgy consisted of 606 nucleotide bases (GenBank accession no. AY929156).

Using RT-PCR, expression of AQP1 and AVT-R genes in various tissues of black porgy acclimated to FW was compared in 10‰ SW and FW. Expression of AQP1 mRNA in black porgy adapted to FW was observed in the pituitary gland, brain, liver and gonad tissues, in addition to gills, kidneys and intestines. These findings were similar to those in reports of expression in the eyes, respiratory organs, kidneys, brain, liver, etc., in mammals (Borgnia et al. 1999; Ma and Verkman 1999). Among AQPs reported so far, AQP 1 functions as a membrane channel peculiar to water movement (Ishibashi et al. 1994, 1997, 2000). Therefore, it can be deduced that AQP1 takes part in water movement in the various tissues of black porgy. AVT-R mRNA expression was also observed by using RT-PCR in various tissues of black porgy adapted to 10‰ SW. In this experiment, weak expression was detected not only in gills and kidneys, but also in the pituitary glands and brain, etc. Therefore, it is deduced that AVT-R action by AVT released from pituitary glands took place in these organs as well. Since AVT-R function in various tissues of fish is yet to be clarified, it is necessary to conduct further studies.

In SW fish, the external osmotic pressure is generally higher than the internal osmotic pressure. Therefore, water loss occurs through the tissues, such as gills, that are highly permeable to water. To replace the water loss, the fish continuously take in SW. The SW is then absorbed into the intestines after salts are removed and ion discharge is activated in the chloride cells of the gills. Also, water re-absorption and cation discharge take place in the kidneys, resulting in reduced urine production (Aoki et al. 2003; Cutler et al. 2007). In this study, AQP1 mRNA expression increased in the osmoregulatory organs of gills, kidneys and intestines when black porgy cultured in seawater was acclimated to 10% SW and FW. It is therefore deduced that water moved into tissues of high permeability, such as gills, by osmotic action, while ion discharge was suppressed in kidneys and intestines and, therefore, water discharge was promoted. Considering that suppression of water re-absorption and production of a large volume of dilute urine in the kidneys of FW-adapted fish are the osmoregulatory functions of kidneys (Cleveland and Trump 1969; Bone et al. 1995; Karnaky 1998), it can be concluded that an imbalance in osmotic pressure of black porgy occurred in the 10‰ SW. Also, it is deduced that AQP1 expression increased because black porgy exposed to a hypoosmotic environment excreted water as a hyperosmoregulatory strategy.

The observation by Martinez et al. (2005) that expression of AQP1 in the kidneys of yellow eels (*Anguilla anguilla* L.) adapted to FW was higher than those adapted to SW was similar to the results of this study. However, in European eels (*A. anguilla*) adapted to SW, AQP1 expression increased in the intestines to become higher than in eels adapted to FW as the volume of absorbed water increased

(Aoki at al. 2003). This leads to the deduction that AQP1 takes part not only in water absorption, but also in water excretion.

The physiological functions that maintain a constant concentration of ions in the body of fish with changes in salinity concentration are largely influenced not only by the related hormones, but also by the degree of expression of hormone receptors in osmoregulatory organs, such as gills, kidneys and intestines. Hormone signals begin functioning as they are delivered into cells with receptors of osmoregulatory organs. Then, proteins become activated in cells to maintain homeostasis of the ion concentrations in each organ (McCormick 2001; Mancera et al. 2002).

In this study, expression of AVT-R mRNA, the AVT receptor taking part in osmoregulation, was observed in gills, kidneys and intestines. Expression of AVT-R mRNA in gills and kidneys was highest in 10% SW and tended to decrease while the fish was adapting to FW. On the other hand, unlike AQP1, AVT-R mRNA expression was not observed in intestines during FW acclimation. Thus, it is deduced that when water permeated into the body of fish, causing imbalance in the osmotic pressure as the external pressure decreased in 10‰ SW, water excretion was promoted in gills and kidneys, but not the intestines, of black porgy for osmoregulation. Also, the decrease in expression afterwards is because the initial level was recovered as black porgy adapted to the hypoosmotic environment during the 24 h in FW. Considering that AVT takes part in both the diuretic and antidiuretic actions of kidneys (Amer and Brown 1995), it was concluded that the osmotic pressure was regulated because AVT-R was activated in gills and kidneys to promote water excretion.

Also, we measured the plasma Na^+, Cl^- and osmolality levels during FW acclimation. These levels were decreased to their lowest levels in FW. It seems to balance ion and water to adapt the hypoosmotic environment. This result is consistent with the previous studies (Mancera et al. 1993; Min et al. 2005).

In conclusion, this study indicated that the expression of AQP1 increased in gills, kidneys and intestines, and the expression of AVT-R increased in gills and kidneys when the SW-cultured black porgy was acclimated to 10‰ SW and to FW. In 10‰ SW, the black porgy were unbalanced in osmoregulation, and then plasma Na^+, Cl^- and osmolality levels were decreased, and it may be that AQP1 and AVT-R genes are synthesized to osmoregulate. And it is thought that the expressions of these genes in FW were decreased because the fish was adapted in some degree to the hypoosmotic environment. From this result, it is deduced that black porgy can hyperosmoregulate through activation of these genes to adapt to the hypoosmotic environments.

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References

Acher R (1996) Molecular evolution of fish neurohypophysial hormones: neutral and selective evolutionary mechanisms. Gen Comp Endocrinol 102:157–172

Acher R, Chauvet J (1995) The neurohypophysial endocrine regulatory cascade: precursors, mediators, receptors, effectors. Front Neuroendocrin 16:237–289

Agre P, Preston GM, Smith BL, Jung JS, Raina S, Moon C, Guggino WB, Nielsen S (1993) Aquaporin CHIP: the archetypal molecular water channel. Am J Physiol 265:F463-F476

Agre P, King LS, Yasui M, Guggio WB, Ottersen OP, Fujiyoshi Y (2002) Aquaporin water channels-from atomic structure to clinical medicine. J Physiol 542:3–16

Amer S, Brown JA (1995) Glomerular actions of arginine vasotocin in the in situ perfused trout kidney. Am J Physiol 269:R775–R780

Aoki M, Kaneko T, Katoh F, Hasegawa S, Tsutsui N, Aida K (2003) Intestinal water absorption through aquaporin 1 expressed in the apical membrane of mucosal epithelial cells in seawater-adapted Japanese eel. J Exp Biol 206:3495–3505

Balment RJ, Wrane JM, Tierney M, Hazon N (1993) Arginine vasotocin and fish osmoregulation. Fish Physiol Biochem 11:189–194

Borgnia M, Nielsen S, Engel A, Agre P (1999) Cellular and molecular biology of the aquaporin water channels. Annu Rev Biochem 68:425–458

Bentley PJ (2002) Endocrines and osmoregulation. Zoophysiology, vol 39. Springer, Berlin

Bone Q, Marshall NB, Blaxter JHS (1995) Biology of fishes, 2nd edn. Blackie Academic and Professional, London

Chang YJ, Hur JW (1999) Physiological responses of grey mullet (*Mugil cephalus*) and Niletilapia (*Oreochromis niloticus*) by rapid changes in salinity of rearing water. J Kor Fish Soc 32:310–316

Chang YJ, Min BH, Choi CY (2007) Black porgy (*Acanthopagrus schlegeli*) prolactin cDNA sequence: mRNA

expression and blood physiological response during freshwater acclimation. Comp Biochem Physiol B 147:122–128

Cho YM, Shin J, Sohn YC (2006) Gene expression levels of growth hormone, Prolactin and their receptors of olive flounder *Paralichthys olivaceus* by salinity changes. J Kor Fish 39:326–332

Chrispeels MJ, Agre P (1994) Aquaporins: water channel proteins of plant and animal cells. Trends Biochem Sci 19:421–425

Cleveland PH Jr, Trump BF (1969) The kidney. In: Hoar WS, Randall DJ (eds) Fish physiology. vol. I: excretion, ionic regulation and metabolism. Academic Press, New York, pp 91–239

Connolly DL, Shanahan CM, Weissberg PL (1998) The aquaporins. A family of water channel proteins. Int Biochem Cell Biol 30:169–172

Conklin DJ, Chavas A, Duff DW, Weaver L, Zhang Y, Olson KR (1997) Cardiovascular effects of arginine vasotocin in the rainbow trout *Oncorhynchus mykiss*. J Exp Biol 200:2821–2832

Cutler CP, Cramb G (2002) Branchial expression of aquaporin 3 (AQP3) homologue is downregulated in the European eel *Anguilla Anguilla* following seawater acclimation. J Exp Biol 205:2643–2651

Cutler CP, Martinez A-S, Cramb G (2007) The role of aquaporin 3 in teleost fish. Comp Biochem Physiol 148: 82–91

Echevarria M, Windhager EE, Frindt G (1996) Selectivity of the renal collecting duct water channel aquaporin-3. J Biol Chem 271:25079–25082

Evans DH (1993) Osmotic and ionic regulation. In: Evans DH (Ed) The physiology of fishes. CRC Press, Boca Raton, pp 315–341

Geering K (1990) Subunit assembly and functional maturation of Na,K-ATPase. J Membr Biol 115:109–121

Harding KE, Warne JM, Hyodo S, Balment RJ (1997) Pituitary and plasma AVT content in the flounder (*Platichthys flesus*). Fish Physiol Biochem 17:357–362

Hatakeyama S, Yoshida Y, Tani T, Koyama Y, Nihei K, Ohshiro K, Kamiie JI, Yaoita E, Suda T, Hatakeyama K (2001) Cloning of a new aquaporin (aqp10) abundantly expressed in duodenum and jejunum. Biochem Biophys Res Commun 287:814–819

Henderson IW, Wales NAM (1974) Renal diuresis and antidiuresis after injections of arginine vasotocin in the fresh water eel (*Anguilla anguilla* L). J Endocrinol 61:487–500

Hirata T, Kaneko T, Ono T, Nakazato T, Furukawa N, hasegawa S, Wakabayashi S, Shigekawa M, Chang M-M, Romero M, Hirose S (2003) Mechanism of acid adaptation of a fish living in a pH3.5 lake. Am J Physiol 284:R1199–R1212

Ishibashi K, Kuwahara M, Gu Y, Tanaka Y, Marumo F, Sasaki S (1998) Cloning and functional expression of a new aquaporin (AQP9) abundantly expressed in the peripheral leukocytes permeable to water and urea, but not glycerol. Biochem Biophys Res Commun 244:268–274

Ishibashi K, Kuwahara M, Kageyama Y, Sasaki S, Susuki M, Imai M (2000) Molecular cloning of a new aquaporin superfamily in mammals. In: Hohmann S, Neilsen S (eds) Molecular biology and physiology of water and solute transport. Kluwer Academic/Plenum Publishers, New York, London, pp 123–126

Ishibashi K, Kuwahara M, Gu Y, Kageyama Y, Tohsaka A, Suzuki F, Marumo F, Sasaki S (1997) Cloning and functional expression of a new water channel abundantly expressed in the testis permeable to water, glycerol, and urea. J Biol Chem 272:20782–20786

Ishibashi K, Sasaki S, Fushimi K, Uchida S, Kuwahara M, Saito H, Furukawa T, Nakajima K, Yamaguchi Y, Gojobori T, Marumo F (1994) Molecular cloning and expression of a member of the aquaporin family with permeability to glycerol and urea in addition to water expressed at the basolateral membrane of kidney collecting duct cell. Proc Natl Acad Sci USA 91:6269–6273

Karnaky JK Jr (1998) Osmotic and ionic regulations. In: Evans DH (ed) The physiology of fishes. CRC Press, Boca Raton, New York, pp 157–176

King LS, Yasui M, Agre P (2000) Aquaporins in health and disease. Mol Med Today 6:60–65

Kitajima C, Tsukashima Y (1983) Morphology, growth and low temperature and low salinity tolerance of sparid hybrids (*Sparus sarba, Acanthopagrus schlegeli*). Jap J Ichthyol 30:275–283

Kinoshita I, Takana M (1990) Differentiated spatial distribution of larvae and juveniles of the two sparids, red and black sea bream in Shijiki Bay. Nippon Suisan Gakkai Shi 56:1807–1813

Kulczykowska E (2001) Responses of circulating arginine vasotocin, isotocin, and melatonin to osmotic and disturbance stress in rainbow trout (*Oncorhynchus mykiss*). Fish Physiol Biochem 24:201–206

Le Mevel J-C, pamantung T-F, Mabin D, Vaudry H (1993) Effect of central and peripheral administration of arginine vasotocin and related neuropeptides on blood pressure and heart rate in conscious trout. Brain Res 610: 82–89

Lignot JH, Cutler CP, Hazon N, Cramb G (2002) Water transport and aquaporins in the European eel (*Anguilla Anguilla*). Symp Soc Exp Biol 49–59

Ma T, Verkman AS (1999) Aquaporin water channels of gastrointestinal physiology. J Physiol 276:F331–F339

Madsen SS, Bern HA (1992) Antagonism of prolactin and growth hormone: impact on seawater adaptation in two salmonids, *Salmo trutta* and *Oncorhynchus mykiss*. Zool Sci 9:775–784

Mahlmann S, Meyerhof W, Hausmann H, Heierhorst J, Schönrock C, Zwiers H, Lederis K, Richter D (1994) Structure, function, and phylogeny of [Arg^8] vasotocin receptors from teleost fish and toad. Proc Natl Acad Sci USA 91:1342–1345

Mancera JM, Perez-Figares JM, 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 JM, Carrion RL, Riodel MDM (2002) Osmoregulatory action of PRL, GH, and cortisol in the gilthead seabream (*Sparus aurata* L.). Gen Comp Endocrinol 129:95–103

Martinez A-S, Cutler CP, Wilson GD, Phillips CPC, Hazon N, Cramb G (2005) Cloning and expression of three

aquaporin homologues from the European eel (*Anguilla anguilla*): effects of seawater acclimation and cortisol treatment on renal expression. Biol Cell 97:615–627

Matsuzaki T, Tajika Y, Tserentsoodol N, Suzuki T, Aoki T, Hagiwara H, Takata K (2002) Aquaporins: a water channel family. Anat Sci Int 77:85–93

McCormick SD (2001) Endocrine control of osmoregulation in fish. Am Zool 282:290–300

Min BH, Kim BK, Hur JW, Bang IC, Byun SK, Choi CY, Chang YJ (2003) Physiological responses during freshwater acclimation of seawater-cultured black porgy (*Acanthopagrus schlegeli*). Korean J Ichthyol 15:224–231

Min BH, Choi CY, Chang YJ (2005) Comparison of physiological conditions on black porgy, *Acanthopagrus schlegeli* acclimated and reared in freshwater and seawater. J Aquacult 18:37–44

Nielsen S, Smith BL, Christensen EI, Agre P (1993) Distribution of the aquaporin CHIP in secretory and resorptive epithelia and capillary endothelia. Proc Natl Acad Sci USA 90:7275–7279

Perrot MN, Carrick S, Balment RJ (1991) Pituitary and plasma arginine vasotocin levels in teleost fish. Gen Comp Endocrinol 83:68–74

Pickford GE, Phillips JG (1959) Prolactin, a factor in promoting survival of hypophysectomized killifish in fresh water. Nature 228:378–379

Pierson PM, Guibboline ME, Mayer-Gostan N, Lahlou B (1995) ELISA measurements of vasotocin and isotocin in plasma and pituitary of the rainbow trout: effect of salinity. Peptides 16:859–865

Preston GM, Agre P (1991) Isolation of the cDNA for erythrocyte integral membrane protein of 28 kilodaltons: member of an ancient channel family. Proc Natl Acad Sci USA 88:11110–11114

Tanaka M, Kimura R, Tagawa M (1991) A thyroxin surge during development of black sea bream larvae and its ecological implication in inshore migration. Nippon Suisan Gakkai Shi 57:1827–1832

Tse WKF, Au DWT, Wong CKC (2006) Characterization of ion channel and transporter mRNA expressions in isolated gill chloride and pavement cells of seawater acclimating eels. Biochem Biophys Res Commun 346:1181–1190

Verkman AS (2002) Aquaporin water channels and endothelial cell function. J Anat 200:617–627

Virkki LV, Cooper GJ, Boron WF (2001) Cloning and functional expression of an MIP (AQP0) homolog from killifish (*Fundulus heteroclitus*) lens. Am J Physiol 81:R1994–R2003

Warne JM (2001) Cloning and characterization of an arginine vasotocin receptor from the euryhaline flounder *Platichthys flesus*. Gen Comp Endocrinol 122:312–319

Warne JM, Balment RJ (1995) Effect of acute manipulation of blood volume and osmolality on plasma [AVT] in seawater flounder. Am J Physiol 269:R1107–R1112

Warne JM, Balment RJ (1997) Changes in plasma arginine vasotocin (AVT) concentration and dorsal aortic blood pressure following AVT injection in the teleost, *Platichthys flesus*. Gen Comp Endocrinol 105:358–364

Warne JM, Bond H, Weybourne E, Sahajpal V, Lu W, Balment RJ (2005) Altered plasma and pituitary arginine vasotocin and hypothalamic procasotocin expression in flounder (*Paltichthys flesus*) following hypertonic challenge and distribution of vasotocin receptors within the kidney. Gen Comp Endocrinol 144:240–247

Watanabe S, Kaneko T, Aida K (2005) Aquaporin-3 expressed in the basolateral membrane of gill chloride cells in Mozambique tilapia *Oreochromis mossambicus* adapted to freshwater and seawater. J Exp Biol 208:2673–2682

Yang B, Verkman AS (1997) Water and glycerol permeabilities of aquaporins 1–5 and MIP determined quantitatively by expression of epitope tagged constructs in Xenopus oocytes. J Biol Chem 272:16140–16146