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Effects of acclimation salinity on the expression of selenoproteins in the tilapia, *Oreochromis mossambicus*

Lucia A. Seale^{a,*}, Christy L. Gilman^a, Benjamin P. Moorman^b, Marla J. Berry^a, E. Gordon Grau^b, and Andre P. Seale^b

^aDept. of Cell and Molecular Biology, John A. Burns School of Medicine, University of Hawaii at Manoa, Honolulu, HI, 96813, USA

^bHawaii Institute of Marine Biology, University of Hawaii, Kaneohe, HI 96744, USA

Abstract

Selenoproteins are ubiquitously expressed, act on a variety of physiological redox-related processes, and are mostly regulated by selenium levels in animals. To date, the expression of most selenoproteins has not been verified in euryhaline fish models. The Mozambique tilapia, *Oreochromis mossambicus*, a euryhaline cichlid fish, has a high tolerance for changes in salinity and survives in fresh water (FW) and seawater (SW) environments which differ greatly in selenium availability. In the present study, we searched EST databases for cichlid selenoprotein mRNAs and screened for their differential expression in FW and SW-acclimated tilapia. The expression of mRNAs encoding iodothyronine deiodinases 1, 2 and 3 (Dio1, Dio2, Dio3), Fep15, glutathione peroxidase 2, selenoproteins J, K, L, M, P, S, and W, was measured in the brain, eye, gill, kidney, liver, pituitary, muscle, and intraperitoneal white adipose tissue. Gene expression of selenophosphate synthetase 1, Secp43, and selenocysteine lyase, factors involved in selenoprotein synthesis or in selenium metabolism, were also measured. The highest variation in selenoprotein and synthesis factor mRNA expression between FW- and SW-acclimated fish was found in gill and kidney. While the branchial expression of Dio3 was increased upon transferring tilapia from SW to FW, the inverse effect was observed when fish were transferred from FW to SW. Protein content of Dio3 was higher in fish acclimated to FW than in those acclimated to SW. Together, these results outline tissue distribution of selenoproteins in FW and SW-acclimated tilapia, and indicate that at least Dio3 expression is regulated by environmental salinity.

Additional keywords

fish; selenium; selenoprotein; acclimation salinity; tilapia; euryhaline

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*Corresponding author: lseale@hawaii.edu, Tel.: (808) 692-1541, Fax: (808) 692-1968.

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1. Introduction

Selenium (Se) is a trace element found in the atmosphere and bodies of water worldwide, originating from both natural and anthropogenic sources [1] and an essential micronutrient to most species, from bacteria to higher vertebrates. Levels of Se are usually reported to be higher in seawater (SW) environments than in freshwater (FW) environments [2]. Selenium is naturally found in inorganic (i.e. selenate and selenite) or organic (i.e. selenomethionine, selenocysteine, dimethylselenide and dimethyldiselenide) forms, and is ultimately incorporated into selenoproteins as the amino acid selenocysteine (Sec). Selenocysteine is encoded by the UGA opal codon and, in eukaryotes, the Sec incorporation mechanism requires several factors to be in place [3], including two selenophosphate synthetases, SPS1 and SPS2 [4], and the protein Secp43 [5]. Decomposition of intracellular Sec is catalyzed by the action of Sec lyase (Scly), which releases selenide to then reenter the selenoprotein synthesis pathway [6].

Because Sec is ionized at physiological pH, most selenoproteins function in strong redox reactions, such as peroxidation and deiodination [7]. Early studies have shown Se-dependent glutathione peroxidase (GPx) activity in fish [8, 9]. The gene for GPx was later inferred in zebrafish (*Danio rerio*) from *in silico* analysis [10], and confirmed experimentally in zebrafish, coho salmon (*Oncorhynchus kisutch*), rainbow trout (*Oncorhynchus mykiss*) and bluefin tuna (*Thunnus maccoyii*) [11–14]. Deiodination is a key step in thyroid hormone activation, and expression of iodothyronine deiodinases has been previously described in various fish species [15], including the euryhaline killifish (*Fundulus heteroclitus*) [16]. In addition to redox reactions, Se is transported through the body via selenoprotein P (Sepp1a), a protein that in zebrafish contains 17 Sec residues [17]. Although other selenoproteins evolved various functions in mammals, including the maintenance of endoplasmic reticulum (ER) homeostasis [18], phospholipid biosynthesis [19], and regulation of gene transcription [20], their function in fish remains unknown.

Interestingly, of the twenty-nine selenoproteins identified in eukaryotes to date, three of them, Fep15, SelJ and SelL are only found in fish [21] or aquatic organisms [18, 22]. In fact, the genomes of aquatic organisms, including fish, encode for more selenoproteins than terrestrial animals [23, 24]. Having a larger selenoproteome suggests that fish have developed greater reliance on environmental Se for redox metabolism. In light of this, it is surprising that little information is available on the abundance and distribution of selenoproteins in fish species that can thrive in environments that vary in salinity and Se content.

The Mozambique tilapia (*Oreochromis mossambicus*) is a euryhaline cichlid fish native to estuarine areas of the coast of Mozambique, eastern Africa [25]. Because this species evolved under exposure to daily tidal changes, it serves as a good model to study opposing osmotic paradigms for survival: a diluting environment in FW and a dehydrating environment in SW [26]. Studies in other fish species have revealed that an increase in environmental salinity can affect the activity of selenoproteins involved in redox reactions [27–29], suggesting that these proteins may play a role in osmotic adaptation. Nevertheless,

these studies did not examine the effects of acclimation salinity in the expression of selenoproteins other than GPx and deiodinases.

To address the possible influence of acclimation salinity on the transcriptional regulation of selenoproteins in a euryhaline fish model, we searched for tilapia selenoproteins in an expressed sequence tag (EST) database. We then screened eight tissues and compared the differences in baseline mRNA expression of twelve selenoproteins and three factors involved in selenoprotein synthesis or Se metabolism in Mozambique tilapia acclimated to SW relative to those acclimated to FW. Based on the availability of antibodies able to detect selenoproteins in fish tissues, we compared the protein levels of Dio3 in FW- and SW-acclimated fish. We also measured the effects of salinity transfers on the branchial gene expression of Dio3.

2. Materials and Methods

2.1. Fish

Mature male Mozambique tilapia (50–100 g), *Oreochromis mossambicus*, were obtained from a population maintained at the Hawaii Institute of Marine Biology, University of Hawaii. Fish were reared in outdoor tanks (700 L) with a continuous flow of FW or SW under natural photoperiod. SW in the tanks was pumped from Kaneohe Bay, Hawaii, USA. SW-acclimated tilapia employed in this experiment were spawned and reared in SW, having never been previously exposed to FW. FW-acclimated tilapia, on the other hand, were spawned and reared in FW, having never been previously exposed to SW. Water temperature was maintained at 24–26 °C. Animals were fed ~5% of their body weight per day with Trout Chow (Skretting, Tooele, UT), which contains ~1 ppm of total Se content according to the manufacturer (personal communication). Fish were fasted for one day prior to sample collection. At the time of sampling, fish were anesthetized with 2-phenoxyethanol (2-PE; 0.3 mL/L). Blood was collected from the caudal vasculature by a needle and syringe coated with ammonium heparin (200 U/mL, Sigma-Aldrich, St. Louis, MO). Plasma was separated by centrifugation and stored at –80 °C for later analyses of Se content. Fish were then rapidly decapitated and the following tissues were collected: brain, eye, gill, kidney, liver, muscle, pituitary and intraperitoneal white adipose tissue (WAT). All tissues were immediately frozen in liquid nitrogen, and subsequently stored at –80 °C prior to RNA extraction or Western blotting analysis. All experiments were conducted in accordance with the principles and procedures approved by the Institutional Animal Care and Use Committee, University of Hawaii.

2.2. Transfer experiments

Mature male Mozambique tilapia (7–55 g) were obtained from a population reared in indoor tanks for 4 months, in either FW or SW. The salinity of one of the FW tanks was switched to SW, and the salinity of one of the SW tanks was switched to FW. It took 1.5 hours for a complete salinity transfer. Fish were fed for the duration of the experiment and water temperature was maintained between 24 and 26 °C. Fish ($n=6-10$) were sampled at 0 h (immediately prior to opening the FW- or SW-valves), 6 h, 24 h, 48 h and 7 days after transfer. At the time of sampling, fish were netted and anesthetized with 2-PE (0.3 mL/L).

Plasma was collected as described above and stored in -20°C until measurement of osmolality. Plasma osmolality was determined using a vapor pressure osmometer (Wescor 5100C, Logan, UT). Filaments from the second gill arch on the left side of the fish were harvested. Gill samples were frozen in liquid nitrogen and stored at -80°C prior to RNA extraction.

2.3. Selenium levels

Selenium content in the tank water, with or without fish, and in the plasma of tilapia was measured by Inductively-Coupled Plasma-Mass spectrometry (ICP-MS), performed by Exova, Inc. (Santa Fe Springs, CA, USA). Briefly, water samples were mixed with 0.5 ml of nitric acid and internal standards, producing 5g of solution for ICP-MS analysis, and assay detection limit was 0.0003 ppm. Plasma sample portions (~ 0.18 g) were mixed with internal standards and then diluted to a mass of 5 g with a solution of 0.1% ammonium hydroxide, 0.05% EDTA and 0.05% Triton X100. After complete dissolution, samples were analyzed by ICP-MS and assay detection limit was 0.02 ppm.

2.4. Extraction of RNA, cDNA synthesis and real-time quantitative PCR (qPCR)

Total RNA was extracted from all tissues with Trizol (Invitrogen, Carlsbad, CA) according to the manufacturer's protocol, followed by purification employing the RNeasy clean-up kit (Qiagen, Valencia, CA). Total RNA concentrations were determined by measuring the absorbance of purified RNA samples at 260 nm wavelength with a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE). Complementary DNA (cDNA) was synthesized by reverse transcription with 1 μg total RNA using the High Capacity cDNA reverse transcription kit (Life Technologies, Carlsbad, CA) according to the manufacturer's instructions. qPCR reactions were carried out on 384-well plates, with a cDNA amount equivalent to 10ng total RNA, 100 nM forward and reverse primers, and SYBR Green PCR Master Mix (Invitrogen, Carlsbad, CA) in a total volume of 5 μl . Cycling conditions were: 2 min at 50°C , 5 min at 95°C followed by 45 cycles at 95°C for 10 sec, 55°C for 10 sec and 72°C for 10 sec. Relative quantification was carried out by the C_t method. Assays were run on a LightCycler 480 system (Roche, Mannheim, Germany).

2.5. Sequences

Sequences for tilapia selenoproteins and selenoprotein synthesis or Se metabolism factors were identified by searching the translated nucleotide online database using a protein query (tBLASTn), at the NCBI Genbank database (<http://www.ncbi.nlm.nih.gov>). The tilapia mRNA sequences for twelve selenoprotein and selenoprotein synthesis factor genes were identified by using templates from known protein sequences of zebrafish; two genes were identified from the protein sequence and cDNA library of Nile tilapia; one was identified from the protein sequence of rainbow trout. The mRNA sequence of SelW was employed as previously published [30]. Accession numbers for each cDNA sequence found after conducting tBLASTn are provided in Table 1. ClustalW program was used for multiple sequence alignments and phylogenetic analyses to ensure sequence conservation among species [31].

2.6. Primers

Primers were designed using the online software, Primer 3 [32] and checked for consensus sequences using Clustal W. Primer sequences and the accession numbers of the cDNAs for primer design are listed in Table 1. All primers were purchased from IDT Technologies (San Diego, CA). Lyophilized primers were resuspended with RNase-free water to a concentration of 100 μ M and further diluted to 10 μ M working stocks. Elongation factor 1 α (EF1- α) was employed as the housekeeping gene used for normalization of all target genes as previously described [33], after it was confirmed that there was no salinity effect on its expression. Specificity and efficiency of all new primers was inferred by performing melting curve analyses, and standard concentration curves, respectively.

2.7. Western Blotting

Gills were lysed using CellLytic MT buffer (Sigma-Aldrich, St. Louis, MO) with protease inhibitors (Calbiochem, Darmstadt, Germany), sonicated in ice bath, centrifuged at 14,000 $\times$ g for 15 min, 4°C, and supernatant was collected. Supernatant protein concentration was determined by the colorimetric Bradford assay using the Bio-Rad protein assay dye reagent (Bio-Rad, Hercules, CA). Samples consisting of 30 μ g of total protein were separated by 4–20% SDS-PAGE (Bio-Rad), transferred to polyvinylidene difluoride membranes (Millipore) and incubated with specific antibody for Dio3 (Novus Biologicals) or β -actin (Cell Signaling) for 1 hour at room temperature, under agitation. Membranes were washed with PBS containing 0.01% Tween-20, incubated with secondary antibodies coupled to infrared fluorophores (Li-Cor Biosciences) for 30 min at room temperature, and blots were visualized using the Odyssey Infrared Imager (Li-Cor Biosciences). The expression of other selenoproteins was not measured due to the lack of specificity of commercially available antibodies.

2.8. Statistical analysis

For each gene and tissue, comparisons between different acclimation salinities were analyzed by Student's *t*-test ($P < 0.05$). Salinity transfer experiments were analyzed by one-way analysis of variance (ANOVA), followed by Fisher's LSD test ($P < 0.05$). Individual values were log-transformed to meet assumptions of normality and equal variance. Statistical calculations were performed using statistical software programs, Prism 5.0 (GraphPad, La Jolla, CA) and Minitab 13.1 (Minitab Inc., State College, PA).

3. Results

3.1. Selenium content

Fresh water employed in this study was found to have non-detectable levels of Se, both in tanks with and without fish. Se content of Kaneohe Bay SW was 0.0049 μ g/g (ppm) and 0.0032 μ g/g (ppm), taken from SW tanks with and without fish, respectively. Plasma Se content was found to be 0.2033 ± 0.014 μ g/g (ppm) in FW-acclimated fish and 0.2175 ± 0.002 μ g/g (ppm) in plasma from SW-acclimated fish. These values were not significantly different from each other ($n=4$, Student's *t*-test).

3.2. Tilapia selenoproteins

Fifteen selenoprotein and selenoprotein synthesis or Se metabolism factor mRNA sequences were identified in the EST database for tilapia. The tilapia mRNA sequences for iodothyronine deiodinases 1, 2 and 3, (Dio1, Dio2 and Dio3), Fep15, glutathione peroxidase 2 (GPx2), selenoprotein J (SelJ), selenoprotein K (SelK), selenoprotein L (Sel L), selenoprotein M (SelM), selenoprotein P (Sepp1a), selenoprotein S (SelS), selenoprotein W (SelW), selenophosphate synthetase 1 (SPS1), Secp43, and Sec lyase (Scly) were found from known protein sequences from zebrafish, Nile tilapia, or rainbow trout. These sequences were highly conserved by comparing consensus regions between different fish species. The sequence for both selenoprotein N (SelN) and thioredoxin reductase 1 (TrxR1) were found in the EST Nile tilapia database (accession numbers GR649079.1 and GR669725.1 for SelN, and GR589931.1 and GR641653.1 for TrxR1) but alignment analysis with the zebrafish sequence (GR694733.1 and AAH54599.1 for SelN and TrxR1, respectively) revealed low similarity; for this reason, SelN and TrxR1 were not included in this study. Other selenoprotein genes, including the expected glutathione peroxidases 1 and 4, were not analyzed due to the lack of available tilapia ESTs at the time of this study.

3.3. Selenoprotein mRNA levels

Differences in selenoprotein mRNA levels between FW and SW-acclimated tilapia were compared in eight tissues (Figure 1, with numerical values in Supplementary Table 1). Sepp1a, SelM and SelS were the only selenoproteins whose mRNA expression was detected in all tissues examined. All three deiodinases (Dio1, Dio2, and Dio3) were most highly expressed in the gill, followed by the brain and the liver (Figure 1A–C). Branchial expression of Dio3 mRNA was higher in FW- than in SW-acclimated tilapia. Expression of Dio1 mRNA was higher in the liver of SW-acclimated fish, while hepatic Dio2 and Dio3 expression did not change according to acclimation salinity. While Dio2 mRNA expression was not detected in the pituitary, Dio1 expression was significantly higher in the pituitary of FW-acclimated fish; pituitary Dio3 expression did not change with salinity.

Gene expression of Fep15 was highest in the brain and kidney. Salinity-dependent differences were only observed in the kidney and liver where gene expression was higher in SW-acclimated fish (Figure 1D). While GPx2 was mostly expressed in the gill, salinity effect was only observed in the kidney, where expression was higher in SW-acclimated fish (Figure 1E). SelJ mRNA expression was highest in the kidney, brain and gill, but a salinity effect was only observed in the brain, where expression was higher in FW-acclimated fish (Figure 1F). SelK was most highly expressed in the gill. A significant effect of acclimation salinity was observed in the muscle, where mRNA levels were higher in SW-acclimated fish (Figure 1G). Although Sel L mRNA levels were greater in the brain there was no salinity effect (Figure 1H).

Gene expression of SelM was highest in the gill, but salinity effects were only observed in the kidney, where mRNA levels were higher in SW-acclimated fish (Figure 1I). Sepp1a was the most highly expressed selenoprotein mRNA in most tissues, with target to housekeeping gene expression ratios of approximately one order of magnitude higher than those of other selenoprotein. Sepp1a expression was higher in the kidney and gill of SW-acclimated fish

when compared with those of FW-acclimated fish (Figure 1J). SelS mRNA expression was highest in the pituitary, and salinity effects were observed in the gill, where expression was higher in FW-acclimated tilapia (Figure 1K). SelW expression was highest in the brain, with higher expression in FW-acclimated fish when compared with SW fish (Figure 1L).

3.4. mRNA expression of selenoprotein synthesis or Se metabolism factors

Genes encoding the factors involved in the selenoprotein synthesis or Se metabolism measured in this study were SPS1, Scly and Secp43 (Figure 2, with numerical values in Supplementary Table 2). Of the three factors, mRNA expression of SPS1 and Scly were the most abundant. SPS1 expression was highest in the gill, followed by the brain. SPS1 levels in the muscle were higher in FW-acclimated fish (Figure 2A). Secp43 mRNA expression was undetected but in the liver, brain, kidney and muscle; no effects of acclimation salinity were observed in any tissue analyzed. Scly mRNA expression was most abundant in the gill, followed by pituitary. Salinity effects were only observed in the kidney, where Scly expression was higher in FW-acclimated fish (Figure 2C).

3.5. Protein levels of Dio3

To characterize whether the effect of acclimation salinity on selenoprotein gene expression was also observed on their gene products, we compared the branchial protein abundance of Dio3 between FW- and SW-acclimated fish. Protein levels of Dio3 were significantly ($P < 0.05$) higher in the gills of FW-acclimated tilapia than in those of SW-acclimated tilapia (Figure 3).

3.6. Effects of salinity transfer on Dio3 gene expression

The effects of salinity transfer on the branchial expression of Dio3 was characterized by collecting gill samples from tilapia at 0 h, 6 h, 24 h, 48 h, and 7 d of transfer from SW to FW and vice-versa. Plasma osmolalities (mean $\pm$ S.E.M.) were 335.3 mOsm/kg $\pm$ 1.87, 315.3 mOsm/kg $\pm$ 1.26, 310.8 mOsm/kg $\pm$ 8.69, 303.6 mOsm/kg $\pm$ 3.73, and 313 mOsm/kg $\pm$ 2.79, at 0 h, 6 h, 24 h, 48 h, and 7 d, respectively, following transfer from SW to FW, and 316.6 mOsm/kg $\pm$ 1.64, 490.9 mOsm/kg $\pm$ 19.05, 434.3 mOsm/kg $\pm$ 14.46, 353.3 mOsm/kg $\pm$ 5.02, and 337.8 mOsm/kg $\pm$ 1.61 at 0 h, 6 h, 24 h, 48 h, and 7 d, respectively, following transfer from FW to SW. Branchial expression of Dio3 increased by 6 h of transfer from SW to FW, but returned to baseline levels throughout the remainder of the transfer period (Figure 4). Consistent with the first experiment, branchial expression of Dio3 was higher in FW- than in SW-acclimated tilapia, when comparing time 0 h of both transfers. Expression of Dio3 declined upon transfer from FW to SW by 6 h and 24 h. By 48 h and 7 d, Dio3 expression was similar to that of the 0 h FW control (Figure 4).

4. Discussion

Although Se levels vary widely among aquatic habitats, little is known on the effects of environmental salinity on selenoprotein mRNA expression in fishes. Using a public EST library database, we were able to identify the genes for twelve expressed selenoproteins and three factors involved in Se metabolism or selenoprotein synthesis, and characterize their expression pattern in eight tissues of Mozambique tilapia reared in either FW or SW since

hatching. We followed up by measuring Dio3 protein content in the gills of fish acclimated to either salinity, and found that the acclimation-salinity effects observed on protein levels follow those observed on branchial gene expression. The effects of salinity transfer on Dio3 gene expression were also measured, revealing both transient and long-term patterns of transcriptional modulation by environmental salinity. To our knowledge, this is the first study to comparatively screen the differential expression between selenoproteins and selenoprotein synthesis factors found in tissues of a euryhaline fish acclimated to either FW or SW, and to characterize the effects of salinity on Dio3 protein levels in the gill.

Previous studies have demonstrated that aquatic organisms have larger selenoproteomes when compared with terrestrial ones. It has been conjectured that a greater diversity of selenoproteins may stem from a life history in aquatic environments [23]. *In silico* analysis using all the available vertebrate genome sequences revealed that the ancestral vertebrate selenoproteome possibly contained twenty-eight selenoproteins, including all the deiodinases, four glutathione peroxidases and two thioredoxin reductases [34]. Only eighteen selenoproteins were previously found in the EST database for zebrafish [10]. Based on the available EST library database, however, we identified twelve of these genes in tilapia and characterized their mRNA expression in response to environmental salinity.

In mammals, the possible molecular mechanisms underlying the effects of Se in the regulation of selenoprotein expression have been discussed [35–38]. An optimal range of Se intake (40–100 µg per day) appears to be required for the maintenance of physiological homeostasis, where deviations from this narrow range increase the probability of health risks, such as Se toxicity or Se deficiency [39, 40]. In fingerling channel catfish (*Ictalurus punctatus*), a daily intake between 0.1 and 0.5 ppm of Se was demonstrated to be ideal for maintenance of liver and plasma GPx activity [41], while in rainbow trout, Se diets containing 0.07 ppm to 4 ppm did not incur signs of Se deficiency nor toxicity [8]. Nonetheless, the daily requirement of Se to prevent Se deficiency or toxicity, and whether this requirement changes with acclimation salinity, is unknown in tilapia. In the present study, although Se levels in SW were higher than those in FW, Se plasma levels in FW- and SW-acclimated tilapia were similar, at around 0.02 ppm. In rainbow trout, liver and kidney Se content was found to be proportional to dietary Se intake [8], and selenomethionine treatment resulted in Se accumulation in the liver, kidney, gills, muscle and brain [42]. The similarity in plasma Se levels between tilapia acclimated to both salinities may be driven by the availability of dietary Se in the commercial chow (~1 ppm), which was three orders of magnitude greater than Se levels found in the water. Thus, the effects of acclimation salinity on selenoprotein expression appear to be independent of circulating Se levels. Albeit in the ppb range, the discrepancy between FW and SW Se levels raises the possibility that selenoprotein expression in response to salinity may be driven by the external concentration of Se. It has been suggested that environmental Se uptake by rainbow trout may take place in FW, where levels are as low as 0.4 ppb [8]. Alternatively, the difference in acclimation salinity *per se*, independent of water Se levels, appears to underlie our observed differences in selenoprotein expression.

Expression patterns and functions of selenoproteins have been most extensively described in mammalian models, with little available information in fish [43]. Our study unveiled the

expression patterns of twelve selenoproteins, including those that are only found in fish and aquatic organisms, such as Fep15, SelJ and Sel L. Fep15, SelK, SelM, and SelS are ER-resident proteins, and thought to share a role in mitigation of ER stress [21, 44]. In mammals, SelK is important for Ca^{2+} flux in macrophages [45], while SelM acts on Ca^{2+} regulation in neurons [46]. We observed SelM and SelK mRNA expression mostly in the gills. Whether these selenoproteins are associated with Ca^{2+} metabolism in the gill remain to be investigated. In the case of SelS, glucose levels were previously shown to inversely regulate its expression in human hepatoma cells [47]. In the present study, SelS increased 5-fold in the gill of FW-acclimated fish compared with SW fish. It is worth noting that while in mammals the brain has high metabolic demand, in fish metabolic demand is high in the gills [48]. Inasmuch as SelS is tied to energy metabolism in fish as it is in mammals, higher levels in the FW gill suggests a difference in branchial metabolic demand between FW and SW-acclimated tilapia.

Selenoprotein J has only been reported in fish and sea urchins. In zebrafish, it was found to form the structural crystallin in the eye lens during development, with broader expression throughout the body in adult stages [22]. Although the expression of SelJ in the eye of tilapia was lower than in most tissues, higher expression in the brain of FW-acclimated tilapia suggests other possible roles for this protein. The biological function of Sel L, which is structurally unique amongst selenoproteins and also restricted to aquatic organisms, is unknown [18]. In the present study, Sel L was most highly expressed in the tilapia brain, and was not affected by acclimation salinity. Sepp1 plays a major role in Se transport and delivery to various organs in the body [49], and in mammals plays an essential role in neurological function [50]. In tilapia, Sepp1 did not change with salinity in the brain, but its role in neurological function remains to be investigated. Expression of SelW, on the other hand, was highest in the brain of FW-acclimated tilapia. Although SelW, which is highly expressed in the developing mammalian brain [51] and is sensitive to changes in Se levels, was one of the first selenoproteins to be identified, little is known about its function. Further studies are required to determine the roles of SelJ, Sel L, Sepp1, and SelW in fish physiology. Together, the differential expression of these selenoproteins between FW and SW-acclimated tilapia suggests that their function may be regulated by acclimation salinity.

We also characterized the tissue distribution of three selenoprotein synthesis and Se metabolism factors in fish acclimated to either FW or SW. Although several mechanistic studies of selenoprotein translation were discovered using zebrafish as a model organism [17, 37, 52], tissue distribution of SPS1, Scly and Secp43 had not been previously reported in teleosts. In the present study, expression of SPS1 was upregulated in the gill of SW-acclimated fish, and muscle of FW-acclimated fish, suggesting that these tissues may respond to salinity by regulating Se metabolism. In mammals, Scly, the enzyme responsible for Se recycling, is mainly expressed and active in the liver and kidney, indicating that reutilization of Se may be associated to hydromineral balance [53]. In the current study, Scly was only upregulated in the kidney of FW-acclimated fish, suggesting that plasma Se levels may be differentially regulated by acclimation salinity with participation of renal mechanisms to maintain plasma Se homeostasis. It must be noted that although total plasma Se concentration was the same between FW and SW-acclimated tilapia, levels of blood-borne Se carrier proteins and molecules, including Sepp1, may vary and affect the

availability of Se for selenoprotein synthesis of various organs according to acclimation salinity.

The tilapia pituitary plays a central role in orchestrating osmoregulatory responses [54–56]. In the present study, Dio1 expression was higher in the pituitary of FW-acclimated fish relative to SW fish. Local conversion of T4 to T3 is performed by deiodinases, which play a key role in thyroid metabolism by locally regulating thyroid hormone availability and activity in peripheral tissues [57]. While Dio1 and Dio2 convert T4 into the active thyroid hormone T3, Dio3 converts T4 to reverse T3 (rT3), which is physiologically inactive. Kinetic deiodination studies in the blue tilapia (*Oreochromis aureus*), have shown that Dio1 is active in the fish brain, where it plays a role in outer ring deiodination [58]. Circulating T3 levels increase after rainbow trout are transferred from FW to SW [29]. Hence, high Dio1 mRNA expression in the pituitary of FW tilapia may be a response to low plasma T3 levels and serve in the regulation of the thyroid hormone negative feedback. In Mozambique tilapia, like in other euryhaline teleosts, gills play an essential role in osmoregulation, by actively uptaking ions in FW and extruding ions in SW [59, 60]. Interestingly, increased branchial Na⁺/K⁺-ATPase (NKA) activity and mitochondria-rich cells size, hallmarks of branchial salinity adaptation, were observed following triiodothyronine (T3) and thyroxine (T4) injections in Mozambique tilapia [61]. It is possible that osmotically-induced local conversion of T4 to T3 followed by the rapid action of T3 on mobilizing ion pumps such as NKA, are major drivers of cellular morphological and functional changes during exposure to increased salinity. Killifish Dio2 has been shown to possess two functional osmotic response elements in its gene promoter [62], which suggests that Dio2 may participate in the osmotic responses in fish. Dio3 mRNA is expressed mainly in the brain and in the gills of Nile tilapia [63]. Although Nile tilapia are less tolerant of high salinity environments than Mozambique tilapia, evidence of Dio3 activity in the tilapia gill [58] suggests that T3 inactivation could play a role in the local regulation of thyroid hormone action during salinity acclimation. In our studies, expression of all deiodinases was higher in the gill of FW-acclimated tilapia than in that of SW fish. This suggests that, through the actions of deiodinases, the local control of T3 availability may be involved in salinity acclimation.

Based on the high expression of selenoproteins in gill and on the availability of antibodies that cross-reacted with selenoproteins in fish, we examined the branchial protein content of Dio3. Consistent with gene expression patterns, protein abundance of Dio3 was higher in the gills of FW-acclimated fish relative to SW fish. These results suggest that, in addition to transcriptional regulation, acclimation salinity may exert a similar effect on protein synthesis of this selenoprotein. Results of transfer experiments also confirmed steady-state acclimation salinity effects on Dio3 mRNA expression. Interestingly, transfer from SW to FW only transiently increased Dio3 expression by 6 h. On the other hand, transfer from FW to SW elicited a transient decrease in the expression of Dio3. Salinity transfers elicit large excursions in plasma osmolality, which in turn triggers mechanisms to restore salt and water balance; such mechanisms are largely mediated by the neuroendocrine system [54–56]. Within days of salinity transfer, deviations in plasma osmolality typically return to baseline levels [64], which range from 305–330 mOsm/kg in FW and 335–360 mOsm/kg in SW (cf. [56]). In the present experiment, changes in Dio3 gene expression in response to salinity

transfers were inversely related to changes in plasma osmolality. Nonetheless, whether these changes in expression are under direct osmotic control or involve the mediation of the endocrine system remain to be investigated.

In conclusion, we found that selenoprotein transcripts in tilapia are ubiquitously expressed, and differentially regulated according to acclimation salinity. This indicates that selenoproteins and their synthesis factors may be involved in osmoregulation and in cellular mechanisms that are sensitive to changes in extracellular osmolality. While these results established a baseline of selenoprotein expression patterns in response to acclimation salinity *in vivo*, further studies are required to elucidate the mechanisms (i.e. hormonal modulation and direct osmotic response) underlying the regulation of selenoproteins and synthesis factors in fish.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Expression of selenoproteins of male Mozambique tilapia acclimated to FW (open bars) or SW (solid bars). Selenoprotein mRNA levels are shown as the ratio of target genes to EF1α. The following genes were measured: Dio 1 (A), Dio2 (B), Dio3 (C), Fep15 (D), GPx2 (E), SelJ (F), SelK (G), Sel L (H), SelM (I), Sepp1a (J), SelS (K), and SelW (L). *, **, *** correspond to significant differences between FW and SW at $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively, using unpaired Student's *t*-test, $n = 6-36$.

Figure 2.

Expression of selenoprotein synthesis factors of male Mozambique tilapia acclimated to FW (open bars) or SW (solid bars). Selenoprotein synthesis factor mRNA levels are shown as the ratio of target genes to EF1α. The following genes were measured: SPS1 (A), Secp43 (B) and Scly (C).*, ** correspond to significant difference between FW and SW at $P < 0.05$ and $P < 0.01$, respectively, using unpaired Student's t -test, $n=6$.

A

Figure 3.

Expression of Dio3 in gill of Mozambique tilapia acclimated to either FW or SW. Dio3 protein intensity levels were normalized by the β -actin levels, and ratios are plotted as average $\pm$ SEM ($n=6$). White bar represents FW-acclimated fish; black bar represents SW-acclimated fish. * $P < 0.05$ by analysis with Student's t-test.

Figure 4.

Changes in Dio3 mRNA expression after transfer of Mozambique tilapia from SW to FW and FW to SW. Bars represent branchial mRNA expression $\pm$ SEM ($n=6-29$). Gene expression is presented relative to the time 0 SW group (black bar). Differences among groups were evaluated by one-way ANOVA, followed by Fisher's LSD test. Means not sharing the same letter are significantly different ($P<0.05$).

Table 1

Primer sequences of selenoproteins and selenoprotein synthesis factors found in the EST database and their respective accession numbers.

Gene	Primers	Amplicon (bp)	GenBank accession numbers (EST)
Dio1	F- AACTATGAGGATTGGGGTCT R- TGAGTCTGGAGCTTCTCCT	117	GR608944.1, GR661324.1
Dio2	F- CTTCTGTTTGTGCGTTTACA R- TTCCAAACACTTTTCTCGTT	132	GR660528.1, GR663328.1
Dio3	F- AGAAACTGGCTGGAACAATA R- ATGGGTGAACATCTGATAGC	160	Y11111.1
Fep15	F- GAGGTGTGTGGATGAAACT R- GCCTCTGACGTACTTGATCT	105	GR672000.1, GR666049.1, GR660980.1, GR609035.1
GPx2	F- GCTGTTGTCTGGAGAACTC R- CTCCTGATGTCCAACTGAT	187	GR660798.1, GR681215.1, GR639987.1, GR701620.1, GR606241.1, GR695782.1
SelJ	F- CCGATAGACAGAATCCTCAG R- GTTGAGTGTGAGGACTCCAT	170	GR632526.1, GR655559.1, GR658891.1, GR605549.1, GR606300.1
SelK	F- TAGAAGCCAAGGAAAGATGA R- ATGAGGGAAGAAGTGATGTG	156	GR638190.1, GR646820.1, GR653210.1, GR649728.1, GR646585.1, GR682968.1, GR650246.1
Sel	L F- GTTCTCCTCAGACATTTTGG R- AAGGTACATCCAGTCTGCTC	161	GR695431.1, GR668977.1, GR666688.1, GR697243.1
SelM	F- TAATGATCTTCGCCTCATTT R- TGCACCTGAGTCTTTTGTG	163	GR611511.1, GR667195.1, GR609923.1, GR621658.1, GR610284.1
SelS	F- GTTCTACCTGCTCATTCTGC R- TCTTCTTGCATCTTCATCCT	138	XM_003459754.1
Sepp1a	F- ATGTTTGGCAGACACTGG R- CCAATGATGGAGTATGGAAG	100	GR619607.1, GR663172.1
SelW	F- GAATATTGTGGTGGATGAGG R- ATAAGGGAATCCACCAGTTT	174	AY737049.1
SPS1	F- GACAAGAACTTCAGGCTCAC R- GGAAGTGTTCATCCTCTTGA	127	GR694970.1, GR676170.1
Sepp43	F- GGGTTACTGTTTGTGGAGA R- GAATACATCTGTCCGCTTTC	153	GR609029.1, GR620171.1, GR700905.1
Sely	F- ATATAGCAGGTGCTAAAGCTAA R- CTCTGCTTTCCTCCAAC	80	GR660440.1, GR669885.1, BJ693872.1