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RESEARCH ARTICLE

Vigorous SO_4^{2-} influx *via* the gills is balanced by enhanced SO_4^{2-} excretion by the kidney in eels after seawater adaptation

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SUMMARY

Sulfate (SO_4^{2-}) is maintained at ~1 mmol l^{-1} in teleost fishes that are exposed to media of varying SO_4^{2-} concentrations. We first measured plasma SO_4^{2-} concentration in euryhaline fishes that adapt to both SO_4^{2-}-poor freshwater (<0.5 mmol l^{-1}) and SO_4^{2-}-enriched seawater (30 mmol l^{-1}). Unlike Mozambique tilapia and chum salmon, Japanese eels maintained higher plasma SO_4^{2-} concentration in freshwater (6.2±2.3 mmol l^{-1}) than in seawater (0.7±0.1 mmol l^{-1}). We then analyzed the whole-body SO_4^{2-} budget using $^{35}SO_4^{2-}$. $^{35}SO_4^{2-}$ influx in seawater-adapted eels occurred by 84.5% *via* body surfaces and 15.5% *via* digestive tracts. The SO_4^{2-} influx was higher in seawater eels (1.55 μmol kg^{-1} h^{-1}) than in freshwater eels (0.09 μmol kg^{-1} min^{-1}), but it was facilitated in freshwater eels when the difference in SO_4^{2-} concentrations between plasma and environment was taken into account (freshwater eels, 6.2 *vs* 0.3 mmol l^{-1}; seawater eels, 0.7 *vs* 30 mmol l^{-1}). One hour after injection of $^{35}SO_4^{2-}$ into the blood of seawater eels, the kidney excreted ~97% of the ionized form, whereas the radioactivity increased gradually in the medium and the rectal fluid more than 3 h after injection. As the radioactivity was poorly adsorbed by anion-exchange resin, $^{35}SO_4^{2-}$ in the blood may be incorporated into cells and excreted by the intestine, gills and skin, probably as mucus. These results show that freshwater eels take up SO_4^{2-} actively from the environment, but seawater eels cope with the obligatory influx of SO_4^{2-} through the gills by excreting excess SO_4^{2-} *via* the kidney and in mucus.

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Key words: marine teleost, sulfate excretion, kidney, eel, ion regulation, *Anguilla japonica*.

INTRODUCTION

Because seawater is a hyperosmotic environment where various ions are dissolved, marine teleost fish continuously cope with a threat of dehydration and excess ion invasion. To compensate for the osmotic loss of water, they drink copious seawater and absorb most of the ingested water *via* the intestine in parallel with monovalent ions such as Na^+ and Cl^- (Smith, 1930; Marshall and Grosell, 2006; Takei and Balment, 2009; Grosell, 2011). The excess Na^+ and Cl^- are excreted actively by mitochondria-rich cells in the gills (Evans, 2008). In contrast to the many studies on Na^+ and Cl^- regulation in fishes, studies on the regulation of divalent ions such as Mg^{2+}, Ca^{2+} and SO_4^{2-} are still limited. In terms of the concentration difference between plasma and environmental seawater, the ratio is 3–4 for Na^+ and Cl^- (Na^+, 170 *vs* 450 mmol l^{-1}; Cl^-, 120 *vs* 500 mmol l^{-1}), but it is 30–50 for Mg^{2+} and SO_4^{2-} (Mg^{2+}, 1 *vs* 50 mmol l^{-1}; SO_4^{2-}, <1 *vs* 30 mmol l^{-1}). Therefore, divalent ions unavoidably enter the body *via* the gills and digestive tracts across the concentration gradient (Hickman, 1968). The excess divalent ions are excreted actively from the proximal tubules of the kidney (Beyenbach et al., 1986; Renfro and Pritchard, 1983).

Sulfate (SO_4^{2-}) is the second most abundant anion in seawater following Cl^-. However, because of the relative difficulty of its measurement, research on SO_4^{2-} regulation, compared with Cl^- regulation, has been largely neglected in marine teleost fish (Marshall and Grosell, 2006). Topologically, the organs that directly contact the external media and have extensive surface area such as the gills and digestive tracts (lumen is an external environment) are possible sites of SO_4^{2-} influx. It is generally accepted that little SO_4^{2-} is absorbed across the intestinal epithelium (Marshall and Grosell, 2006), but Hickman (Hickman, 1968) estimated that 11.3% of SO_4^{2-} derived from ingested seawater is absorbed by the intestine of southern flounder (*Paralichthys lethostigma*). Concerning the gills, SO_4^{2-} permeability has been examined in freshwater teleosts and variable data were reported: significant permeability was found in the guppy (Rosenthal, 1961), whereas little permeability was detected in the goldfish (Garcia and Maetz, 1964). However, detailed analyses of the SO_4^{2-} budget between body fluids and media have not yet been reported in euryhaline fish that experience profound changes in SO_4^{2-} concentration between freshwater and seawater.

To collect basic information about the whole-body SO_4^{2-} budget in teleost fish, we used Japanese eels (*Anguilla japonica* Temminck and Schlegel 1846) as a model because they adapt readily to both freshwater and seawater, and various techniques for *in vivo* experiments have been established. Initially, we measured plasma SO_4^{2-} and other ion concentrations in eels adapted to freshwater or seawater and compared the data with those of other euryhaline species, Mozambique tilapia [*Oreochromis mossambicus* (Peters 1852)] and chum salmon [*Oncorhynchus keta* (Walbaum 1792)]. We found that SO_4^{2-} concentration in freshwater eels was much higher than those of tilapia and chum salmon, as reported previously by Nakada et al. (Nakada et al., 2005). However, the plasma SO_4^{2-} concentration in seawater eels was even lower than in tilapia and salmon, showing suppressed influx and/or enhanced excretion of

SO_4^{2-} ions. We thus compared SO_4^{2-} concentrations in the urine and rectal fluid between freshwater and seawater eels to estimate the site of SO_4^{2-} excretion. Further, we examined the whole-body SO_4^{2-} regulation in detail by measuring the uptake and excretion of SO_4^{2-} at the regulatory sites (gills/skin, intestine and kidney) using $^{35}SO_4^{2-}$ as a tracer in conscious, catheterized eels. As radioactivity appeared immediately in the urine but slowly in the media and rectal fluid after tracer injection, we determined whether the material secreted into the media and the rectal fluid is ionized $^{35}SO_4^{2-}$ using anion-exchange resin.

MATERIALS AND METHODS

Animals

Cultured Japanese eels (192±5 g, *N*=39) were purchased from a local dealer. Fifteen eels were maintained in freshwater tanks and 24 eels were maintained in seawater tanks for more than 2 weeks before use. Chum salmon (109±4 g, *N*=12) and tilapia (125±3 g, *N*=12) were reared in either freshwater or seawater for more than 1 month. Temperatures were maintained at 18°C for eels, 12°C for chum salmon and 25°C for tilapia. Eels were not fed after purchase, and chum salmon and tilapia were starved for 5 days before experiments. The ionic compositions of freshwater and seawater were as follows: 1.0 mmol l^{-1} Na^+, 0.5 mmol l^{-1} K^+, 0.5 mmol l^{-1} Cl^-, 0.25 mmol l^{-1} Ca^{2+}, 0.5 mmol l^{-1} Mg^{2+} and 0.3 mmol l^{-1} SO_4^{2-} for freshwater; and 450 mmol l^{-1} Na^+, 12.5 mmol l^{-1} K^+, 525 mmol l^{-1} Cl^-, 10 mmol l^{-1} Ca^{2+}, 50 mmol l^{-1} Mg^{2+} and 30 mmol l^{-1} SO_4^{2-} for seawater. All conditions for fish maintenance were in accordance with the Guidelines for Animal Care and Maintenance at University of Tokyo and the experiments were approved by the Bioscience Committee of the University of Tokyo.

Comparison of plasma ions among euryhaline teleosts

Plasma ion concentrations (Na^+, Cl^-, SO_4^{2-}, Ca^{2+} and Mg^{2+}) and osmolality were measured in eels, tilapia and salmon and compared between freshwater- and seawater-acclimated fish. The ion concentrations were also measured in the urine and rectal fluid of freshwater- and seawater-acclimated eels (*N*=9 each). Fluid samples were collected from the caudal vein, urinary bladder and rectum after anesthesia in 0.1% (w/v) tricaine methanesulfonate (Sigma-Aldrich, St Louis, MO, USA). Blood samples without anticoagulant were centrifuged immediately after collection at 10,000 ***g*** for 5 min at 4°C to separate plasma. Ionized anions and cations were measured by ion chromatography (AV10, Shimadzu, Kyoto, Japan) using a cation-exchange column (IC-C3) and an anion-exchange column (IC-A3).

Measurement of SO_4^{2-} influx

Freshwater and seawater eels (*N*=6 each) were anesthetized as above and the ventral aorta was catheterized as described previously (Tsuchida and Takei, 1998). A schematic drawing of the experimental set up is shown in supplementary material Fig. S1. After more than 18 h of recovery from surgery, eels were transferred to a bucket with 2 l of freshwater or seawater, and 7.4 MBq of $^{35}SO_4^{2-}$ (1.59 TBq mg^{-1}, Muromachi Science, Tokyo, Japan) was added to the medium. Urine and 75 µl of blood were collected 0, 1, 3, 6, 12 and 24 h after the isotope administration, and 25 µl of plasma or urine was mixed with 5 ml of scintillation cocktail (AQUASOL-2, PerkinElmer, Shelton, CT, USA) for measurement in a scintillation counter (LS6000 SC, Beckman, Fullerton, CA, USA).

To evaluate the role of the digestive tract in SO_4^{2-} uptake, six seawater eels were cannulated as above, and the esophagus was intubated with a polyethylene tube (0.61 mm o.d.). Seawater was infused through the tube into the esophagus at a constant rate (1 ml h^{-1}) to maintain water balance in seawater (Takei, 2000). After more than 18 h of recovery, three eels were transferred to a bucket with 2 l of seawater containing 7.4 MBq of $^{35}SO_4^{2-}$ and infused seawater into the esophagus, and the other three eels were transferred to $^{35}SO_4^{2-}$-free seawater and infused seawater containing 3.7 MBq l^{-1} $^{35}SO_4^{2-}$. Blood was collected as above for measurement of radioactivity.

Measurement of SO_4^{2-} efflux

After anesthesia, the ventral aorta and urinary bladder of seawater eels (*N*=6) were catheterized as described previously (Renfro and Pritchard, 1983) using a vinyl tube (1.5 mm o.d.) inserted into the rectum through the anus. A schematic drawing of the experimental setup is shown in supplementary material Fig. S1. After more than 18 h after surgery, 1.7 MBq of $^{35}SO_4^{2-}$ was injected into the ventral aorta in a volume of 100 µl for 30 s followed by a flush with 100 µl of isotonic saline. The dead volume of the tube was ca. 30 µl. Then 500 µl of medium in the bucket, whole urine and rectal fluid, as well as 75 µl of blood were collected 0.5, 1, 3, 6, 12 and 24 h after $^{35}SO_4^{2-}$ administration for measurement of radioactivity.

To separate the ionic $^{35}SO_4^{2-}$ from ^{35}S containing metabolites, collected fluids were incubated with activated anion-exchange resin (DEAE Sephadex™ G-25, GE Healthcare, Uppsala, Sweden) at 25°C for 3 h. The mixture was centrifuged at 10,000 ***g*** for 15 min at 4°C. The absorptive capacity of the resin was confirmed by cold SO_4^{2-}. Supernatant (10 µl for plasma, urine and rectal fluid and 50 µl for the medium) was mixed with 5 ml of scintillation cocktail for measurement of radioactivity.

Calculation of SO_4^{2-} fluxes

Extracellular fluid volume of the Japanese eel was estimated to be 15% of body mass based on the Cl^- space and Na^+ space of European eel (Kirsch, 1972a; Mayer and Nibelle, 1969). The time course of $^{35}SO_4^{2-}$ influx was highly linear (r^2=0.98 for seawater eels and r^2=0.96 for freshwater eels, P<0.01) for 24 h after administration of isotope to the medium. In contrast, the disappearance curve of $^{35}SO_4^{2-}$ from the circulation was fitted best to a dual exponential function ($A_t=ae^{-\alpha t}+be^{-\beta t}+c$), where A_t is plasma radioactivity at time t, and a, b, c, α and β are constants (Takei and Hatakeyama, 1987). The efflux rate of $^{35}SO_4^{2-}$ was calculated based on the specific activity of $^{35}SO_4^{2-}$ in the plasma. Curve fitting was performed using statistical software (KyPlot 5.0, Kyens, Tokyo, Japan).

Statistical analyses

Ionic concentrations of body fluids and flux rates were compared between freshwater and seawater fish using Student's *t*-tests and Tukey–Kramer tests, respectively. Influx rate was analyzed by linear regression. All analyses were performed using KyPlot 5.0 software. Significance was defined at P<0.05. All results are expressed as means ± s.e.m.

RESULTS

Plasma ion concentrations of euryhaline teleosts in freshwater and seawater

Plasma ionic concentrations and osmolality were generally higher in seawater fish than in freshwater fish irrespective of species (chum salmon, Mozambique tilapia and Japanese eel), except for SO_4^{2-} concentration in freshwater eels (Fig. 1). The higher values for Na^+, Cl^-, Mg^{2+}, Ca^{2+} and osmolality in seawater fish were more prominent in eels than in tilapia or salmon, particularly plasma Cl^- concentration. Unlike salmon and tilapia, plasma SO_4^{2-}

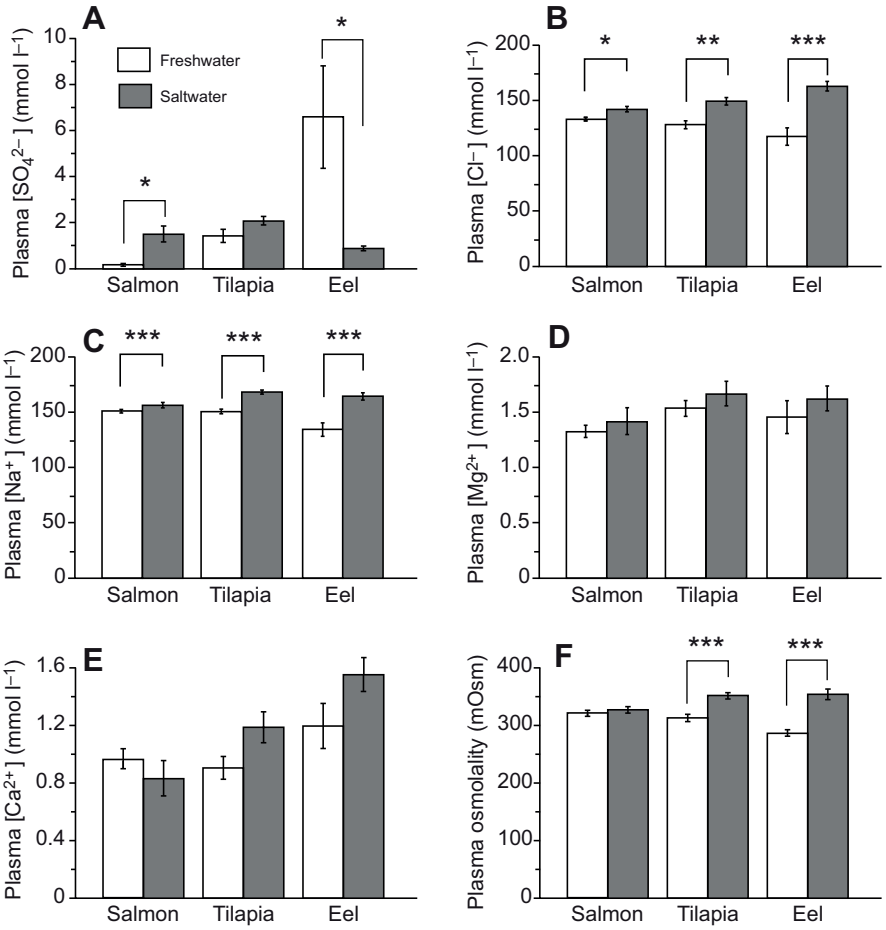

Fig. 1. Plasma SO_4^{2-} (A), Cl^- (B), Na^+ (C), Mg^{2+} (D) and Ca^{2+} (E) concentrations and osmolality (F) in freshwater- and seawater-acclimated chum salmon (*N*=6 each), Mozambique tilapia (*N*=6 each) and Japanese eel (*N*=9 each). Asterisks indicate significant differences between freshwater and seawater (**P*<0.05; ***P*<0.01; ****P*<0.001).

concentration was suppressed to ~1 mmol l^{-1} in seawater eels (Fig. 1A). Urine SO_4^{2-} concentration was 40-fold higher in seawater eels than in freshwater eels, indicating active excretion of SO_4^{2-} by the kidney (Fig. 2). SO_4^{2-} concentration in the rectal fluid was 25% higher in seawater eels than in freshwater eels.

SO_4^{2-} influx in freshwater and seawater eels

Plasma radioactivity increased linearly after administration of $^{35}SO_4^{2-}$ to the medium in both seawater and freshwater eels, and the influx rate was calculated to be 1.55±0.21 μmol kg^{-1} h^{-1} for seawater eels (*N*=6) and 0.09±0.03 μmol kg^{-1} h^{-1} for freshwater eels (*N*=6) after correction of specific activity of $^{35}SO_4^{2-}$ in each medium (Fig. 3A). To take into account the difference in the SO_4^{2-} concentrations between plasma and the medium of freshwater (6.2 *vs* 0.3 mmol l^{-1}, 20.6 times) and seawater eels (0.7 *vs* 30 mmol l^{-1}, 0.02 times), we corrected the influx value at each time point after administration of $^{35}SO_4^{2-}$. The rate was reversed and more facilitated in freshwater eels (1.76±0.21 μmol kg^{-1} h^{-1}) than in seawater eels (0.03±0.01 μmol kg^{-1} h^{-1}) after correction (Fig. 3B). When the influx was compared between intact eels and esophagus-ligatured eels, the rate decreased by ~15% (Fig. 4). Furthermore, when esophagus-ligatured eels in $^{35}SO_4^{2-}$-free seawater were infused with $^{35}SO_4^{2-}$-containing seawater through the esophagus at the normal drinking rate, the increase in plasma radioactivity was ~15% compared with intact eels in $^{35}SO_4^{2-}$-containing seawater (Fig. 4). Based on these data, SO_4^{2-} influx was calculated to be 84.5% from the body surfaces (gills and skin) and 15.5% from the digestive tracts (esophagus, stomach and intestine).

SO_4^{2-} excretion in seawater eels

Plasma radioactivity gradually decreased after injection of $^{35}SO_4^{2-}$ into the blood of seawater eels (Fig. 5A). Urine radioactivity quickly increased within 1 h after injection, and a high level was maintained for 6 h followed by a gradual decrease (Fig. 5B). Radioactivity in the rectal fluid remained low for 3 h and increased linearly thereafter up to 24 h (Fig. 5C), indicating secretion of metabolic products containing ^{35}S into the intestinal lumen. Radioactivity in the medium also remained low for almost 6 h and then increased linearly (Fig. 5D), again suggesting secretion of ^{35}S-containing metabolites from the gills and skin. In fact, 85.6% of radioactivity in urine was identified as $^{35}SO_4^{2-}$, as the radioactivity was adsorbed to anion-exchange resin (Table 1). However, only 32.3% in rectal fluid and 10.1% in medium was adsorbed by the resin. Therefore, using the data from 30 min to 1 h in Fig. 5, the excretion rate of ionic SO_4^{2-} was calculated to be 1.56±0.18 μmol kg^{-1} h^{-1} by the kidney, 0.05±0.01 μmol kg^{-1} h^{-1} into the intestinal lumen and 0.0011±0.0008 μmol kg^{-1} h^{-1} into the medium by the gills and skin in seawater eels (*N*=6). This result shows that 96.8% of ionic SO_4^{2-} was excreted *via* the kidney of seawater eels.

DISCUSSION

Marine teleost fish are continually faced with an excess of SO_4^{2-} that permeates the body surfaces according to the concentration gradient imposed by the high SO_4^{2-} concentration in environmental seawater. The major site of influx may be the gills because they have an extensive surface area and separate the blood from environmental seawater only by a thin monolayer of respiratory cells

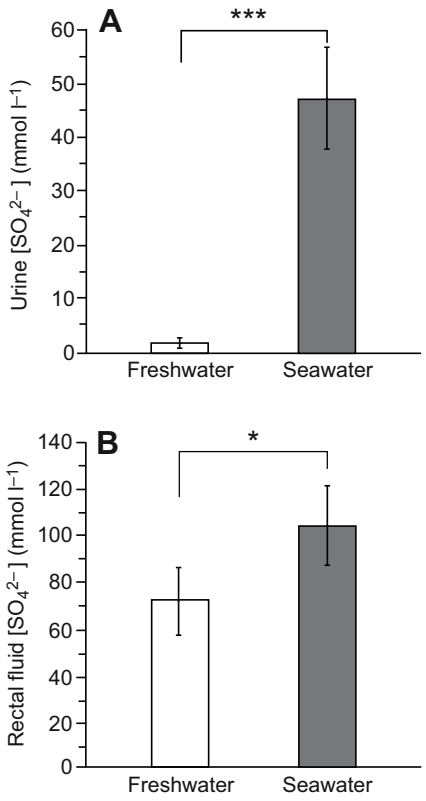

Fig. 2. SO_4^{2-} concentrations in the urine (A) and rectal fluid (B) of freshwater and seawater eels (N=9 each). Asterisks indicate significant differences between freshwater and seawater (*P<0.05; ***P<0.001).

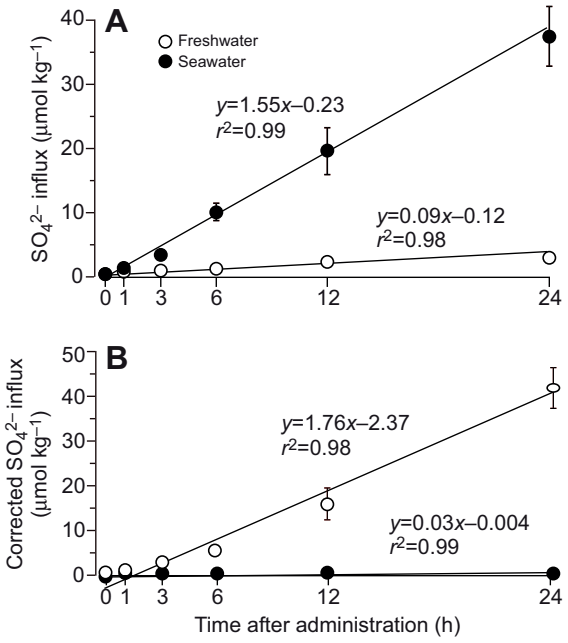

Fig. 3. (A) Time-course changes in SO_4^{2-} influx into the blood of freshwater- (N=6) and seawater-acclimated eels (N=6). (B) SO_4^{2-} influx corrected by taking into account the difference in SO_4^{2-} concentrations between plasma and media (freshwater, 6.2 *vs* 0.3 mmol l^{-1}; seawater, 0.7 *vs* 30 mmol l^{-1}). Regression equations and their corresponding correlation coefficients are shown in the figure.

(Evans, 2008; Marshall and Grosell, 2006). The SO_4^{2-} excess is further exaggerated by intestinal absorption due to copious drinking of environmental seawater in marine teleosts (Hickman, 1968; Takei and Balment, 2009). However, SO_4^{2-} transport activity is generally very low in the intestine of marine teleosts (Marshall and Grosell, 2006). The secretion of excess SO_4^{2-} is accomplished in part by its active secretion at the proximal tubules of the kidney, as evidenced by high SO_4^{2-} concentration in the urine compared with other anions (Renfro and Pritchard, 1983; Dickman and Renfro, 1986; Cliff and Beyenbach, 1992). Based on these previous data, the following conclusions can be drawn by the present study: (1) freshwater eels maintain high plasma SO_4^{2-} concentrations (6.2 mmol l^{-1}) by facilitated SO_4^{2-} uptake from the medium (17.7 μmol kg^{-1} h^{-1}); (2) seawater eels offset obligatory SO_4^{2-} influx *via* the gills by enhanced SO_4^{2-} excretion by the kidney and maintain low plasma SO_4^{2-} concentrations (0.7 mmol l^{-1}); and (3) a part of SO_4^{2-} fluxed into the body of seawater eels was also excreted by the gills, skin and digestive tracts as mucus and other metabolic products.

High plasma SO_4^{2-} concentrations in freshwater eels

Nakada et al. (Nakada et al., 2005) were the first to report unusually high plasma SO_4^{2-} concentrations in freshwater eels (~20 mmol l^{-1}). The high value may be exaggerated, as the plasma Cl^- concentration of the eels is extremely low (~70 mmol l^{-1}). In this study, we measured a plasma SO_4^{2-} concentration of freshwater eels of 6.2 mmol l^{-1}, which was much higher than that of the other euryhaline teleosts examined (Mozambique tilapia and chum salmon). The SO_4^{2-} concentration was lower in freshwater than in seawater tilapia and salmon, but the relationship was reversed in eels, where plasma SO_4^{2-} concentration was suppressed to ~1 mmol l^{-1}. The SO_4^{2-} concentrations in the urine and rectal fluid of seawater eels were also comparable to those of other marine teleost fishes (Berglund and Forster, 1958; Hickman, 1968; McDonald and Grosell, 2006). These results clearly show that, in eels, profound changes occur in SO_4^{2-} regulation when they move from freshwater to seawater.

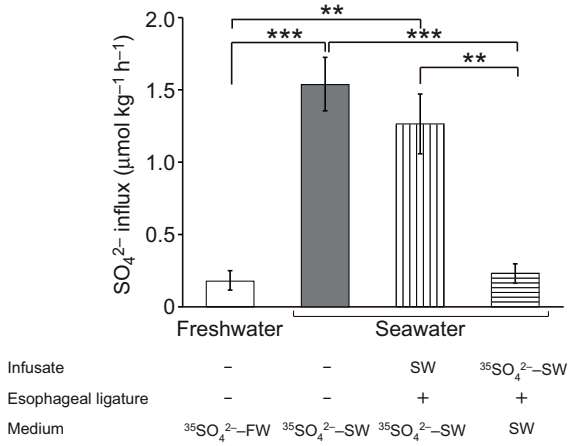

Fig. 4. SO_4^{2-} influx rate in freshwater eels in $^{35}SO_4^{2-}$-enriched freshwater (FW; N=6), seawater eels in $^{35}SO_4^{2-}$-enriched seawater (SW; N=6), seawater eels with esophageal ligature in $^{35}SO_4^{2-}$-enriched seawater (N=3), and seawater eels with esophageal ligature in $^{35}SO_4^{2-}$-free seawater with an infusion of $^{35}SO_4^{2-}$-enriched seawater into the esophagus (N=3). Treatments are shown below the figure. Asterisks indicate significant differences between treatments (**P<0.01; ***P<0.001).

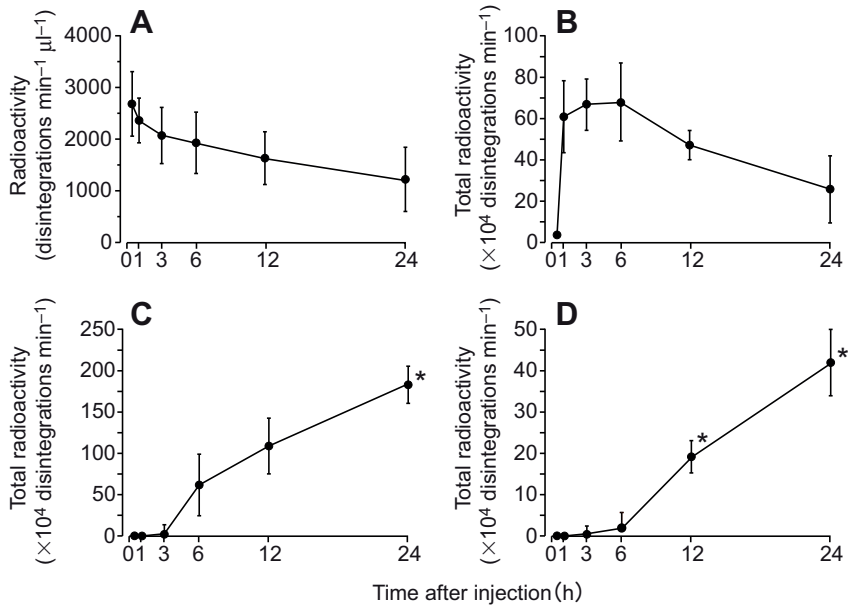

Fig. 5. Time-course changes in radioactivity of (A) plasma, (B) urine, (C) rectal fluid and (D) medium after injection of $^{35}SO_4^{2-}$ into the blood of seawater-acclimated eels (N=6). Values for plasma indicate concentration; all others are total radioactivity. The changes were fitted to a dual exponential function as shown in the figure. Asterisks indicate significant differences between freshwater and seawater (*P<0.05).

Using the eel as a model, we recently found that in seawater, Cl^-, not SO_4^{2-}, is responsible for switching the SO_4^{2-} regulation from a freshwater-retention type to a seawater-extrusion type (Watanabe and Takei, 2011a).

In the plasma of Mozambique tilapia and chum salmon, not only SO_4^{2-} but also other ions (Na^+ and Cl^-) were higher in seawater than in freshwater. In eels, plasma Na^+ and, particularly, Cl^- concentrations were also higher in seawater than in freshwater. Because the sum of negative charges (Cl^- + SO_4^{2-}) appears to be similar among different species, SO_4^{2-} may compensate for the lack of negative charge caused by low plasma Cl^- concentration in freshwater eels. In fact, the total negative charge of Cl^- and SO_4^{2-} was almost the same in three euryhaline fishes in freshwater and seawater in the present study (data not shown), and plasma Cl^- was inversely correlated with plasma SO_4^{2-} concentration (Watanabe and Takei, 2011b). Thus, freshwater eels appear to have an unusually high tolerance to hypersulfatemia or a unique mechanism to neutralize the toxic effects of excess SO_4^{2-}.

Tracer experiments using $^{35}SO_4^{2-}$ showed that the influx rate of SO_4^{2-} was 1.55 µmol kg^{-1} h^{-1} in seawater eels, which is balanced by active excretion by the kidney at a rate of 1.56 µmol kg^{-1} h^{-1}. Comparing the flux rates of ions, the rate for SO_4^{2-} influx is much lower than that of Na^+ (13.2 mmol kg^{-1} h^{-1}) and Cl^- (0.48 mmol kg^{-1} h^{-1}) in seawater eels (Kirsch and Meister, 1982; Tsukada et al., 2005), as in other marine teleosts (Marshall and Grosell, 2006). Therefore, it seems that SO_4^{2-} transport across the body surfaces is suppressed in seawater eels compared with Na^+ and Cl^-. In fact, the turnover rate of Na^+ and Cl^- is greatly accelerated in euryhaline teleosts when they are in seawater compared with freshwater (Kirsch, 1972b; Miyazaki et al., 1998), probably because their influxes counter osmotic water loss by the gills and their absorption increases water uptake by the intestine (Marshall and Grosell, 2006).

SO_4^{2-} influx from the environment

The results obtained from the esophagus-ligatured seawater eels revealed that 84.5% of SO_4^{2-} influx occurred *via* the body surfaces and only 14.5% entered *via* the intestine. The major site of influx in the body surface may be the gills, as the eel skin is covered by small scales and mucus and its surface area is 1/20 that of the total area of the gill surface (Motais and Isaia, 1972). It has been suggested that the intestine of marine teleosts is almost impermeable to SO_4^{2-}, which allows changes in luminal SO_4^{2-} concentration to be used as a marker for water absorption along the intestine (Marshall and Grosell, 2006). However, the present study showed that intestinal absorption of SO_4^{2-} is significant in seawater eels.

The SO_4^{2-} influx in the gills and intestine of seawater eels may be regulated by the transcellular and intercellular pathways through transporters located on the apical and basolateral membranes of epithelial cells. Concerning the transcellular pathway, little is known about SO_4^{2-} transporters and channels in the gills. In the intestine, solute carrier (Slc) 26a6 family members, anion exchangers that potentially transport SO_4^{2-}, have been identified in the eel (Watanabe and Takei, 2011b) and pufferfish (Kurita et al., 2008). However, Slc26a6 localized on the apical membrane of epithelia may secrete SO_4^{2-} into the lumen in exchange for Cl^-, as has been shown in the proximal tubule of seawater eel kidney (Watanabe and Takei, 2011a). It was also shown that Slc26a6 in the seawater fish intestine is involved in the secretion of HCO_3^- into the lumen for $CaCO_3$ precipitation to decrease luminal fluid osmolality (Grosell, 2011). Slc26a1, which also

Table 1. Rate of ionic SO_4^{2-} secretion in Japanese eels 24 h after $^{35}SO_4^{2-}$ injection as shown by treatment with anion-exchange resin

	Pre-treatment	Post-treatment	
	Amount secreted (µmol kg^{-1} h^{-1})	Amount adsorbed (µmol kg^{-1} h^{-1})	Free $^{35}SO_4^{2-}$ (%)
Urine	24.2±1.92	20.4±0.72	85.6±4.13
Rectal fluid	46.8±4.56	15.1±1.44	32.3±1.33
Medium	48.5±3.12	4.80±0.24	10.1±0.90

Values are means ± s.e.m.

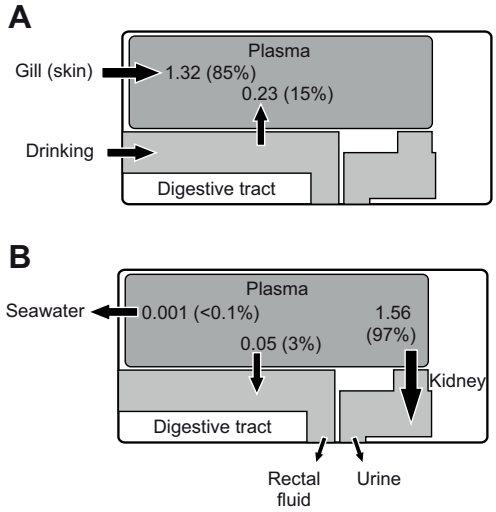

Fig. 6. Schematic drawing of the sites responsible for SO_4^{2-} influx (A) and efflux (B) between the external medium and the plasma of seawater-acclimated eels. Values are an average of six fish and are expressed in $\mu mol\,kg^{-1}\,h^{-1}$. Relative values (%) among the sites are given in parentheses.

transports SO_4^{2-}, was localized abundantly in the basolateral membrane of proximal tubule of eel kidney (Nakada et al., 2005; Watanabe and Takei, 2011b) and in the kidney of other teleost fish (Katoh et al., 2006; Kato et al., 2009), but its gene transcripts were detectable only in the rectum of seawater eels (Watanabe and Takei, 2011b). Judging from the fact that the gills and intestine of seawater eels take up SO_4^{2-}, unknown SO_4^{2-} transporters are likely to be present in these tissues. A possible candidate is Slc26a3, which is briskly expressed in the anterior intestine of seawater eels and is a major SO_4^{2-} transporter in the intestine of mammals (Markovich, 2001); however, its physiological role is not known in fish.

As SO_4^{2-} concentration in seawater is more than 40-fold greater than that of plasma, the paracellular pathway is a probable route of SO_4^{2-} uptake. However, the uptake is influenced not only by the concentration gradient but also the transepithelial potential difference. It has been reported that the transepithelial potential is +23 mV (inside positive) in seawater killifish (Wood and Grosell, 2008). If this is the case also in eels, then both the concentration gradient and the transepithelial potential facilitate SO_4^{2-} uptake through the intercellular route. In fact, the influx rate of SO_4^{2-} is $1.55\,\mu mol\,kg^{-1}\,h^{-1}$ in seawater eels.

For freshwater fish, the gills may also be a major site of SO_4^{2-} uptake; for example, eels usually drink little in freshwater and thus intestinal absorption may be negligible (Takei, 2000). The influx rate of SO_4^{2-} of freshwater eels from the medium into the body was as low as $0.09\,\mu mol\,kg^{-1}\,h^{-1}$ in the present study. However, because the plasma SO_4^{2-} concentration was more than 20-fold higher than that of environmental freshwater, SO_4^{2-} was lost across the concentration gradient but uptake occurred in freshwater. Further, transepithelial potential was shown to decrease to –39 mV in killifish after transfer to freshwater, which also impedes SO_4^{2-} uptake from the medium (Wood and Grosell, 2008). Thus, it is obvious that SO_4^{2-} influx is much enhanced in freshwater eels, although the actual influx rate is much smaller than that in seawater eels.

SO_4^{2-} efflux into the environment

In the present study, $^{35}SO_4^{2-}$ injected into the plasma was secreted immediately into the urine but not into the intestinal lumen or into the medium. Thus, ionized SO_4^{2-} is secreted mostly (~97%) by the kidney within 1 h after injection. Consistently, 85.1% of the radioactivity was adsorbed to the anion-exchange resin in the urine collected even after 24 h of tracer injection. Previous studies have shown that the secretion of SO_4^{2-} is achieved at the proximal tubules of the kidney in several teleost species (Katoh et al., 2006; Kato et al., 2009; Watanabe and Takei, 2011a), in which apical Slc26a6 and basolateral Slc26a1 localized on the same cell play major roles in SO_4^{2-} transport from blood into the tubular lumen. However, ionized SO_4^{2-} was excreted into the intestinal lumen only by 32.5%. The gene transcripts of Slc26a6a, b and c and Slc26a1 were detected in the eel intestine but in a different segment (Watanabe and Takei, 2011a). Both Slc26a6a and Slc26a1 mRNA were detected only in the rectum of seawater eels (Watanabe and Takei, 2011a), where SO_4^{2-} may be secreted into the lumen in an ionic form. The ionic SO_4^{2-} secreted from the gills and skin was only 10% as judged by the adsorption to the anion-exchange resin.

Significant amounts of $^{35}SO_4^{2-}$ injected into the circulation was taken up by the tissues, processed, and secreted into the medium and rectal fluid more than 3 h after injection. This indicates that $^{35}SO_4^{2-}$ was incorporated into some metabolic products and secreted from the gills, skin and intestine. In mammals, mucus is made from various sulfated proteins and sugars (Amerongen et al., 1998). Marine teleosts have large amounts of sulfated glycoproteins in abundant goblet cells in the digestive tract and mucus cells in the gills and skin, as shown by Alcian Blue staining (Sarasquete et al., 2001). Thus most ^{35}S may be incorporated into the mucus. Because ^{35}S-radioactivity in the rectal fluid increased to a significant amount after 24 h (Fig. 5C), the role of intestine in the secretion of excess SO_4^{2-} seems to be important for maintenance of low plasma SO_4^{2-} concentration in seawater eels. In mammals, sulfotransferase is capable of converting glycoprotein into sulfated substances including mucin (Brockhausen, 2003), but such a pathway has not been demonstrated in fishes.

Fig. 6 summarizes the relative contribution of osmoregulatory sites to the whole-body SO_4^{2-} budget in seawater eels as shown by this study. It is obvious that the vigorous influx of SO_4^{2-} *via* the gills across the concentration gradient is nullified by active renal excretion. Recently, we localized several Slc transporters in the apical and basolateral membrane of epithelial cells in different segments of the renal proximal tubule in seawater eels (Watanabe and Takei, 2011a). Our next target may be to identify molecules and the mechanism of SO_4^{2-} transport in the gills and intestine of seawater eels and those concerned in the active uptake of SO_4^{2-} by the gills of freshwater eels. Because eels are unique in their SO_4^{2-} regulation among euryhaline fishes, they provide an interesting model for identifying important regulatory mechanisms in teleost fish.

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