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Changes in activity and transcript level of liver and gill metabolic enzymes during smoltification in wild and hatchery-reared masu salmon (*Oncorhynchus masou*)

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ABSTRACT

It is important for the success of the masu salmon, *Oncorhynchus masou*, stock enhancement program in Hokkaido (northern Japan) to demonstrate physiological problems in hatchery-reared (hatchery) smolt for artificial release. The present study examined changes in liver and gill metabolic parameters in wild and hatchery masu salmon during smoltification and elucidated differences in hepatic and gill metabolism between wild and hatchery fish. As reference to freshwater-adapted wild and hatchery smolt in this study, metabolic parameters of coastal smolt were studied. Yearling wild and hatchery smolting fish were collected from the Ken-ichi River and the Donan Research Branch, which used Ken-ichi river water for fish culture, at the same time every month from March through May 2008. Coastal smolts were caught from Nemuro Bay of Hokkaido in June. Decreased hepatic glycogen content during smoltification, which was observed in wild fish and revealed activation of glycogenolysis, was not found in hatchery fish. Hatchery fish demonstrated a positive change in hepatic ATP content during smoltification, while wild fish showed negative change in the content, which reflected activated consumption of hepatic ATP stores during smoltification. Increases in gill pyruvate kinase activity during smoltification, which were found in wild fish and indicated activation of glycolysis, were not detected in hatchery fish. There was a difference in increased timing of hepatic citrate synthase activity during smoltification between hatchery and wild fish. Increased gill citrate synthase activity during smoltification, which was observed in wild fish and reflected enhancement of the citric acid cycle, was not found in hatchery fish. Hatchery smolt revealed lower liver cytochrome *c* oxidase activity and transcript levels of some respiratory chain enzymes compared to wild smolt in May, which suggested lower respiratory chain capacity in hatchery fish at mid-smolt stage. On the other hand, there were no remarkable differences in hepatic and gill 3-hydroxyacyl-coenzyme A dehydrogenase related to lipolysis and creatine kinase activities, which operate in resolution of creatine phosphate, during smoltification between hatchery and wild fish. These results suggested hatchery masu salmon had some metabolic problems with carbohydrate metabolism, the citric acid cycle, and the respiratory chain. Our study will give valuable information to improve physiological quality of hatchery smolt for artificial release.

1. Introduction

Masu salmon (*Oncorhynchus masou*), which are distributed in the western Pacific countries, are an important coastal fishery resource in Hokkaido, northern Japan. In Hokkaido, almost all anadromous masu salmon undergo smoltification, which is a complex of physiological and biochemical changes to be adaptive for life under seawater, and downstream migration in spring at age 1. Artificial propagation, primarily through release of yearling hatchery-reared (hatchery) smolts into the river, has been conducted as a stock enhancement program for masu salmon, but the coastal masu salmon stock does not show consistent increases in northern Japan. Success of the propagation depends on releasing hatchery smolt with high physiological quality. In hatchery smolts, which are produced at the Hokkaido Fish Hatchery, seawater adaptability is as high as wild smolts (Mizuno et al., 2004). Therefore, it is necessary to clarify if other physiological problems exist in hatchery smolts that may impact smolt success.

Changes in metabolism during smoltification and transfer from freshwater to seawater have been demonstrated in metabolic enzyme activity, the amount of metabolites, and metabolic rate in Atlantic

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salmon (*Salmo salar*), coho salmon (*Oncorhynchus kisutch*), chinook salmon (*Oncorhynchus tshawytscha*), and rainbow trout (*Oncorhynchus mykiss*). In Atlantic salmon, standard and active metabolic rate is higher in smolts compared to parr (Maxime et al., 1989). Atlantic salmon smolts have lower hepatic and muscle glycogen (GC) and increased blood glucose (GL) level compared with parr (Wendt and Saunders, 1973). Sheridan et al. (1985) demonstrated that decreased hepatic GC content was caused by a combination of reduced GC synthesis and increased glycogenolysis in coho salmon smolts. Muscle phosphofructokinase activity, a glycolytic enzyme, was elevated during smoltification in Atlantic salmon (Leonard and McCormick, 2001). The activities of several key enzymes of glycolysis increase during adaptation from freshwater to seawater in the gill, liver, and muscle of large rainbow trout (Soengas et al., 1995a,b,c). Total body and muscle lipid content decreased during smoltification in Atlantic salmon (Saunders and Henderson, 1978). Lipid depletion during smoltification depends on increased lipolysis and decreased fatty acid synthesis in coho and chinook salmon (Sheridan et al., 1985; Sheridan, 1988; Cowley et al., 1994). Citrate synthase activity, a citric acid cycle enzyme, of liver, gill, and kidney was enhanced during smoltification in Atlantic salmon (McCormick et al., 1989b), but it did not change during seawater transfer (McCormick et al., 1989a). In the gill and liver, activity of respiratory enzymes increased during smoltification in Atlantic salmon (Langdon and Thorpe, 1985; McCormick and Saunders, 1987). However, there was little information on metabolism of masu salmon smolt, on metabolism of seawater-adapted smolt, and on gene expression of metabolic enzymes in smolts. The present study examined the activity and transcript level of several metabolic enzymes in wild and hatchery juveniles during smoltification and coastal smolt in masu salmon.

2. Materials and methods

2.1. Fish

Yearling smolting wild masu salmon were collected by electrofishing (Model 12-B Backpack Electrofisher, Smith-Root, Vancouver, BC, Canada) in the Hiyamizu Stream, which is tributary of the Ken-ichi River, in southwestern Hokkaido on March 4 (4.6 °C), April 3 (5.3 °C), and May 1 (9.8 °C), 2008. There were no hatchery fish in the Hiyamizu Stream from February 8 to May 1, because release of the hatchery smolts was performed on May 2, 2008. Yearling hatchery smolting juveniles were captured using electrofishing in the Donan Research Branch of Hokkaido Fish Hatchery, which utilizes water from the Ken-ichi River for fish culture, on the same time schedule as collection of smolting wild fish. Smolt phases (Kato, 1991) of both wild and hatchery masu salmon in March, April, and May were characterized as parr, pre-smolt, and mid-smolt, respectively. Coastal smolts, i.e., post-smolts, were captured by two-boat surface trawling in Nemuro Bay of eastern Hokkaido at latitude 43°31′N, 145°19′E on June 4, 2008, since it was accepted that hatchery smolts released from the Ken-ichi River in May had previously been observed in the Nemuro Bay in early June. However, it is unknown whether the captured fish are of hatchery or wild origin. Water temperature and salinity at the coastal smolt sampling were 9.7 °C and 32‰, respectively.

2.2. Sampling

Captured fish were measured for fork length (FL) and body weight (BW) after anesthesia using 300 mg/L 2-phenoxyethanol. Condition factor (CF) was calculated as $CF = 100 \times BW\ (g)/FL\ (cm)^3$. Blood was collected using a 1.5-mL test tube by decaudation in smolting wild and hatchery fish except for coastal smolts. In sampling of coastal smolts, blood was rejected after removing the tail. Serum was separated from blood by centrifugation at 1.0×10^4 rpm for 15 min at 4 °C and frozen at −85 °C until serum GL analysis. The entire gill arch assembly on both the right and left sides and the whole liver were sampled after blood sampling, and all tissues except for the first gill arch on the left side were frozen at −85 °C. Gill lamellae were removed from the first gill arch on the left side, and frozen and preserved in SEI buffer (0.3 mol/L sucrose, 20 mmol/L EDTA, 0.1 mmol/L imidazole) at −85 °C for analysis of Na^+,K^+-ATPase activity.

2.3. Assays

The assay for gill Na^+,K^+-ATPase activity was routinely performed using 96-well microplates according to the method of Riley et al. (2008). In brief, the first gill arch on the left side was homogenized in 750 μL of SEI buffer with deoxycholic acid (2.41 mmol/L) and centrifuged at 2.5×10^3 rpm for 8 min. Ten-microliter samples were run in two triplicate sets: one set containing assay mixture and the other assay mixture and 0.72 mmol/L ouabain. After reaction for 10 min at 37 °C, released inorganic phosphate (Pi) was determined spectrophotometrically at 630 nm using microplate reader (Tosou, Tokyo, Japan). The resulting ouabain-sensitive ATPase activity was expressed as micromole Pi per milligram protein per hour. Protein concentrations were determined with the BCA protein assay kit (Pierce, Rockford, IL, USA).

Serum was used for determination of GL concentration using commercial GL test kits according to the mutarotase-GOD method (Wako Pure Chemical, Osaka, Japan). Assays for liver GC and triglyceride (TG) content were performed according to Misaka et al. (2004). A piece of sampled liver for GC analysis was homogenized in heated 30% (w/v) potassium hydroxide, added to ethanol, and centrifuged at 2.5×10^3 rpm for 20 min at 4 °C. The precipitate, which was added to 2 mol/L HCl and 1 mol/L H_2SO_4 in turn, was heated for 2 h to decompose GC into GL. After the heated sample was centrifuged at 2.5×10^3 rpm for 20 min at 4 °C, GL content of the supernatant was analyzed using the GL test kit (Wako Pure Chemical) and expressed as percentage of GC content/liver (w/w). A piece of liver sample was homogenized with ethanol/diethyl ether (3:1, v/v) and centrifuged at 2.5×10^3 rpm for 20 min at 4 °C. TG content of the supernatant was examined using TG test kit (Wako Pure Chemical) and shown as percentage of TG content/liver (w/w).

For ATP content and metabolic enzyme assays, a part of liver and the first gill arch on the right side, were homogenized with ice-cold 20 volumes homogenization buffer (5 mmol/L HEPES, 1 mmol/L EDTA, 0.01% Triton X-100, pH 7.4). Homogenates were then centrifuged at 1.5×10^4 rpm for 20 min, and the supernatants were used for assays. ATP content analysis was performed according to Burness et al. (2005). Luciferin-luciferase (dissolved in 100 mmol/L glycine, 20 mmol/L $MgSO_4$, pH 7.4) was added to the sample. Total amount of luminous intensity for 5 min was measured using luminescence reader (BLR310, Aloka, Tokyo, Japan). The ATP standard ranged between 10^{-16} mol/L and 10^{-8} mol/L. ATP content was expressed as picomole ATP per gram wet tissue.

Metabolic enzyme assayed at 25 °C using UV/Visible spectrophotometer (Ultrospec 2000; GE Healthcare UK, Buckinghamshire, UK) under the following specific condition, which referred to Moyes et al. (1997) and Burness et al. (2005).

2.3.1. Pyruvate kinase (PRK)

The assay contained 5 mmol/L ADP, 100 mmol/L KCl, 10 mmol/L $MgCl_2$, 0.15 mmol/L NADH, 10 μmol/L fructose-1,6-bisphosphate, 5 mmol/L phosphoenolpyruvate, and excess lactate dehydrogenase (free of PRK) in 50 mmol/L 3-(*N*-morpholino) propanesulfonic acid (MOPS; pH 7.4). The assay was started with enzyme at 340 nm but was strictly dependent on phosphoenolpyruvate.

2.3.2. 3-Hydroxyacyl-coenzyme A (CoA) dehydrogenase (HOAD)

The assay contained 0.1 mmol/L acetoacetyl CoA, 0.15 mmol/L NADH and 0.05% Triton X-100 in 50 mmol/L PIPES (pH 7.0). The assay