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Changes in glycogen concentration and gene expression levels of glycogen-metabolizing enzymes in muscle and liver of developing masu salmon

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ABSTRACT

Glycogen, as an intracellular deposit of polysaccharide, takes important roles in energy balance of many animals. In fish, however, the role of glycogen during development is poorly understood. In the present study, we assessed changes in glycogen concentration and gene expression patterns of glycogen-metabolizing enzymes in developing masu salmon (*Oncorhynchus masou masou*), a salmonid species inhabiting west side of North Pacific Ocean. As we measured glycogen levels in the bodies and yolk sacs containing the liver separately, the glycogen concentration increased in both parts as the fish developed, whereas it transiently decreased in the yolk sac after hatching, implying glycogen synthesis and breakdown in these tissues. Immunofluorescence staining using anti-glycogen monoclonal antibody revealed localization of glycogen in the liver, muscle and yolk syncytial layer of the pre-hatching embryos and hatched larvae. In order to estimate glycogen metabolism in the fish, the genes encoding homologs of glycogen synthase (*gys1* and *gys2*) and glycogen phosphorylase (*pygma*, *pygmb* and *pygl*) were cloned, and their expression patterns were assessed by quantitative PCR and *in situ* hybridization. In the fish, *gys1* and *gys2* were robustly expressed in the muscle and liver, respectively. Also, expression of *pyg* isoforms was found in muscle, liver and yolk syncytial layer during hatching. With changes in glycogen concentration and expression patterns of relevant genes, our results suggest, for the first time, possible involvement of glycogen in energy balance of fish embryos, especially during hatching.

1. Introduction

Glucose is the most important monosaccharide as a substrate for energy production in animals. Circulating glucose in blood or extracellular space can be absorbed by the cells and readily utilized to produce ATP following glycolysis and, in case oxygen is available, aerobic respiration in mitochondria (McIlwain and Tresize, 1956; Wasserman, 2009). In many tissues such as liver, muscle and brain, glucose can be stored in the form of glycogen. Whereas liver glycogen is often used to increase blood glucose levels, most glycogen in other tissues is locally consumed to meet cellular energy demands after food deprivation, exercise, hypoxia, *etc.* (For reviews, see Adeva-Andany et al., 2016; Waitt et al., 2017). Synthesis and degradation of glycogen in tissues are appropriately controlled to condition cellular and organismal energy balance. Among processes of glycogen synthesis, glycogen synthase (Gys) is known as a key enzyme. In most vertebrate animals, there are two isoforms of Gys: Gys1 is ubiquitously expressed but mainly in the muscle, while Gys2 is specifically expressed in the liver. Similarly, glycogen breakdown is carried out with a rate-limiting enzyme, glycogen phosphorylase (Pyg), the isoforms of which, Pygm, Pygl, and Pygb, being primarily expressed in muscle, liver, and brain, respectively. In adult fish, the mechanisms of glycogen metabolism with above enzymes are comparable to those in mammals (Pereira et al., 1995; Chang et al., 2007; Cruz et al., 2010), although glycogen utilization during starvation is rather modest in fish (French et al., 1981; Blasco et al., 1992). Decades ago, presence of glycogen in salmonid yolk-feeding embryos and larvae was reported (Daniel, 1947; Walzer and Schönenberger, 1979a, 1979b). Since then, however, few studies have focused on glycogen as possible energy reservoir in developing teleosts: proteins and lipids are much richer in egg yolk, attracting researchers in this context (Kamler, 1992). Because fish embryos and larvae tend to show higher mortalities than juveniles and adults, energy

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metabolism during these critical periods needs more insights for establishment of better aquaculture, breeding, and seedling production systems. Recently, increasing evidences showed that glucose takes indeed an essential role in developing fish (Gut et al., 2013; Rocha et al., 2015; Marandel et al., 2016), leading to our attention to glycogen as potential energy reservoir contributing to development of fish.

Here, we report glycogen metabolism in developing masu salmon (*Oncorhynchus masou masou*), an anadromous salmonid species distributed in west side of the North Pacific Ocean, with special reference to the liver and muscle. First, we assessed changes in glycogen levels of yolk sacs (containing the liver) and bodies, separately, of the fish along developmental courses until nearly end of yolk absorption. Since glycogen content in yolk sacs and bodies showed dynamic changes, we further cloned the genes encoding homologs of Gys and Pyg, and assessed their spatiotemporal expression patterns. Changes in glycogen levels found in developing masu salmon, especially before hatching, may reflect possible involvement of glycogen in development of teleost fish.

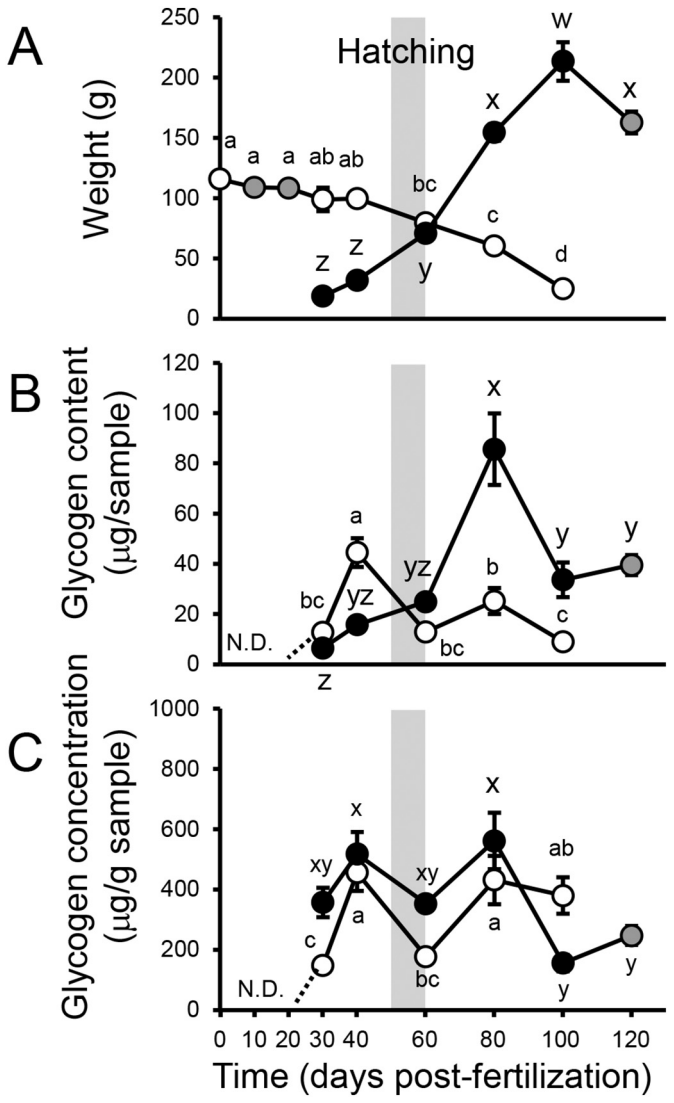
2. Materials and methods

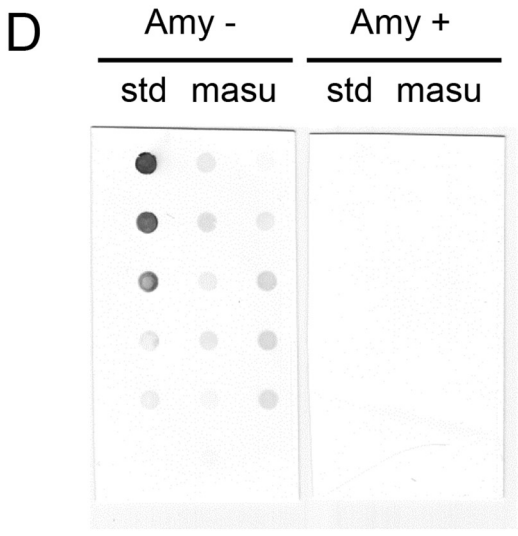
2.1. Sampling

Cultured female and male fish (four each) were selected and separately spawned by pressing ventral side of their bodies, and part of the eggs was fertilized at a commercial aquaculture company, Yamame No Sato, Gokase-town, Miyazaki, Japan. The unfertilized eggs were immediately sampled at Yamame No Sato, and fertilized eggs were then carried to University of Miyazaki, where the eggs were transferred into baskets sunk in recirculating freshwater (8.5 °C) in a 100-L tank. The water was aerated, and refreshed with dechlorinated tap water several times after hatching, which took place 55–65 days post-fertilization (dpf). The developing masu salmon embryos and larvae were sampled at 10, 20, 30, 40, 60, 80, 100, and 120 dpf for subsequent analyses. At 60 dpf, only hatched fish were sampled. The fish were anesthetized with 0.1% 2-phenoxyethanol before sampling, and for those unhatched (10–40 dpf), the chorions were manually removed by fine forceps before anesthesia. For the fish at 30–100 dpf, the yolk sacs were removed from the bodies, and these parts were assayed separately. Since the liver was attached with the yolk sac, with vitelline vessels connecting each other, values for the yolk and liver were measured together (yolk + liver). For glycogen measurement, the samples were put in tubes, weighed, snap-frozen in liquid nitrogen, and stored at −20 °C. RNA samples were preserved by treating tissues in RNAlater solution (Qiagen, Hilden, Germany) and stored in −80 °C, following the manufacturer's instructions. For *in situ* hybridization and glycogen localization, samples were fixed in 4% paraformaldehyde (PFA) in phosphate-buffered saline (PBS) overnight at 4 °C, transferred to methanol, and then stored at −20 °C. The experiments were conducted in accordance with the guidelines in University of Miyazaki and Kitasato University, and every effort was made to minimize the number of animals sacrificed and their suffering.

2.2. Glycogen measurement

Glycogen samples were prepared and measured according to Dreiling et al. (1987) and Baba (1993), respectively, with slight modifications. To prevent degradation of glycogen, the samples were generally handled on ice. Tissues were homogenized in 500 µL of 8% perchloric acid (PCA) and then centrifuged at 14,000 rpm for 10 min at 4 °C. The supernatant was transferred to another tube, mixed with equal volume of petroleum ether, centrifuged again, and the bottom aqueous layer was aliquoted and stored at −20 °C for glycogen assay. At glycogen measurement, the samples were thawed, diluted 1:50 with 8% PCA, and combined with PBS and methanol in proportion of samples: PBS: methanol = 1:7:2. Then the samples were blotted onto PVDF





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