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Research Paper

Effect of severe environmental thermal stress on redox state in salmon

Toshiki Nakano *, Masumi Kameda [1], Yui Shoji [2], Satoshi Hayashi [3], Toshiyasu Yamaguchi, Minoru Sato

Marine Biochemistry Laboratory, Graduate School of Agricultural Science, Tohoku University, Aoba-ku, Sendai, Miyagi 981-8555, Japan

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ABSTRACT

Fish are exposed to many kinds of environmental stressors and the chances of succumbing to infectious diseases may be increased a result. For example, an acute increase in temperature can induce numerous physiological changes in the body. In the present study, we examined the redox state in response to a severe acute stress resulting from heat shock in teleost coho salmon (*Oncorhynchus kisutch*). The plasma lipid peroxides levels in fish gradually increased after heat shock treatment. By 2.5 h post-heat stress, plasma glutathione (GSH) levels had decreased, but they had returned to basal levels by 17.5 h post-stress. Plasma superoxide dismutase activities in stressed fish were significantly increased compared with those in control fish at 17.5 h post-stress, but had returned to basal levels by 48 h post-stress. Expression levels of hepatic GSH and heat shock protein 70 gradually increased after heat shock treatment. These results concerning the changing patterns of multiple important redox-related biomarkers suggest that severe thermal stressors can affect the redox state and induce oxidative stress in ectothermal animals, such as fish, *in vivo*. Hence, manipulation of appropriate thermal treatment may possibly be useful to control fish fitness.

Introduction

Aquatic organisms are exposed to local and global environmental stressors, such as pollutants and acute changes in temperature [1–6]. Exposure of organisms to stressors may result in a series of biochemical and physiological changes. At the organismal level, these changes are mediated by the neuroendocrine system. In addition to this neuroendocrine stress response, there is a cellular stress response following exposure to stressful situations. These stress responses in organisms affect their general health, disease resistance, growth, and reproduction [3,5–7]. An acute increase in temperature is known as heat shock and can induce numerous changes in the body. The physiological states of fish depend on the environmental temperature. As a result, temperature is an important factor influencing their biological geographic distribution. Furthermore, daily and seasonal temperature changes have an impact during the lifetime of individual fish [8]. Unfortunately, studies on the heat shock response in fish have primarily focused on the expression and characterization of cellular molecular chaperons, heat shock proteins (HSPs) [1,3,8–10].

Recently, in the course of studies on the fish fitness in response to a stress, we found that mild stress caused by handling as an acute physiological stressor regulates the expression of growth-related genes, such as growth hormone receptor (*ghr*) and insulin-like growth factor-1 (*igf1*) genes, in fish [11]. The growth of fish is known to be genetically regulated and to be also influenced by cellular, endocrinological, and environmental factors. The responses of endocrine tissue are affected by the integration of external stimuli with internal signals according to the physiological state [1,3,5–7,12–16]. Fish growth can be enhanced by improved nutrition, husbandry conditions, elevated temperature, and changes in the endocrine system of the animal [5,15,17]. Accordingly, it is of interest to determine the effects of severe stressors on the expressions of important genes such as growth-related genes in fish. It is needed to reveal the features of and the resulting effects of stressors on fish fitness in order to improve their production and health. In addition, fish are thought to be an ideal and a convenient model to examine the effects of thermal and other complex stressors on the organism for both short and long periods. This is because fish are a typical ectothermic vertebrate. However, little is known about the effects of acute increases in temperature, which should be severe stressors for fish, on conditions such as the redox state in fish.

In the present study, we examined the redox state in response to a severe stress derived from heat shock in teleost coho salmon (*Oncorhynchus kisutch*). Coho salmon is one of the most valued

* Corresponding author.
E-mail address: nakanot@bios.tohoku.ac.jp (T. Nakano).
[1] Present address: Tochigi Prefectural Government Office, 1-1-20 Hanawada, Utsunomiya, Tochigi 320-8501, Japan.
[2] Present address: Sendai City Construction Office, 3-20-2 Oritate, Aoba-ku, Sendai, Miyagi 982-0261, Japan.
[3] Present address: Nagano Prefectural Hokushin Regional Office, 955 Hekida, Nakano, Nagano 383-8515, Japan.

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species used in aquaculture worldwide and is known to be susceptible to increases in temperature [18]. Additionally, in Japan, coho salmon farming is one of the basic industries in the north-eastern (Tohoku) Pacific coastal area, where the great earthquake and massive tsunami occurred in 2011. We discuss the relationships between the thermal stress responses and the redox states in fish in the context of our findings.

Materials and methods

Animal experiment

All experimental procedures were approved by the Animal Care Committee at Tohoku University (Sendai, Japan). Coho salmon *O. kisutch* were purchased from a local hatchery (Miyagi, Japan). After acclimatization for 2 weeks at the Aquarium Facility of Tohoku University, fish were exposed to heat shock (+11 °C for 2 h) and sampled at 2.5, 17.5, and 48 h after stress.

The fish (approximate body weight, 144 g) were reared in 60-L flow-through tanks at 8 °C (light/dark=12 h/12 h). Healthy, mixed sex, fish were divided into 4 groups ($n=8$). The fish in the first group were undisturbed (prestressed) fish used as a control, maintained under quiet and suitable conditions, and sampled at 13:30. The fish in the second group were subjected to heat shock from 9:00 to 11:00 and sampled at 2.5 h post-stress (at 13:30). The fish in the third group were subjected to the stressor from 18:00 to 20:00 and sampled at 17.5 h post-stress (at 13:30). The fish in the fourth group were also subjected to the stressor from 11:30 to 13:30 and sampled at 48 h post-stress (at 13:30). Accordingly, all tissues and blood for analysis were sampled at the same time, so that the effects of several factors, such as diurnal rhythm and photoperiod, on the expressions of redox state-related factors could be minimized.

Food was withheld for over 48 h before each sampling period. At each sampling period (2.5, 17.5, and 48 h post-stress), fish were sacrificed by an overdose of buffered MS222 (m-aminobenzoic acid ethyl ester methanesulfonate). Blood was withdrawn and plasma was separated by centrifugation. Fish were gutted, and the tissues were quickly removed. All plasma and tissue samples were frozen at −80 °C until analysis.

Measurements

Plasma cortisol and glucose levels

Plasma cortisol levels were measured using an enzyme-linked immunosorbent assay (ELISA) kit from Oxford Biomedical Research, UK [19]. Plasma glucose was measured using an enzymatic assay method with a Glucose CII-Test Wako kit from Wako Pure Chemical Industries, Ltd., Japan.

Lipid peroxides, glutathione, superoxide dismutase, and heat shock protein 70 levels

Lipid peroxides (LPO) were determined as thiobarbituric acid reactive substances (TBARS) by a HPLC-fluorescence method [20]. TBARS concentrations were determined from a standard curve established with TBA-malondialdehyde (MDA, 1,1,3,3-tetramethoxypropane) adducts.

Glutathione (GSH) levels were determined by a glutathione reductase-recycling method with a Total Glutathione Quantification kit from Dojindo Laboratories, Japan. This kit can measure the total amount of reduced GSH and oxidized form of GSH (GSSG).

Superoxide dismutase (SOD) activity was assayed by the formazan-WST method (Total SOD Assay kit, Dojindo Laboratories, Japan).

Levels of HSP70 protein were determined by immunoblotting as described by Basu et al. (2001) [19]. Anti-HSP70 and anti-β-actin antibodies were purchased from Sigma-Aldrich, St. Louis, MO. The expressions of HSP70 were normalized by those of β-actin.

Protein contents were measured by a DC Protein Assay kit (Bio-Rad Laboratories, Hercules, CA) using bovine serum albumin as standard.

Statistical analysis

All samples were run in duplicate and results were expressed as means ± SEM. All data were subjected to one-way analysis of variance (ANOVA). Means were compared with the Tukey–Kramer multiple comparison test. Differences were considered to be statistically significant at $p<0.05$.

Results

Plasma cortisol and glucose levels

At 2.5 h post-heat stress, plasma cortisol levels had increased compared with those in control fish, but had returned to basal levels at 17.5 h post-stress (Fig. 1). At 2.5 and 17.5 h post-stress, plasma glucose levels had increased as compared with those in control fish (the average plasma glucose concentration in control fish was 66.4 mg/dL). However, at 48 h post-stress, plasma glucose levels in stressed fish had decreased and were not significantly different from those in control fish (data not shown).

Plasma LPO, GSH, and SOD levels

As shown in Fig. 2A, the plasma LPO levels in stressed fish gradually increased after heat shock treatment, and had significantly increased compared with those in control fish at 17.5 and 48 h post-stress.

At 2.5 h post-heat stress, plasma GSH levels had decreased, but had returned to basal levels at 17.5 h post-stress (Fig. 2B). At 48 h post-stress, plasma GSH levels in stressed fish had increased significantly as compared with those in control fish.

Plasma SOD activities in stressed fish had increased significantly compared with those in control fish at 17.5 h post-stress, but had returned to basal levels at 48 h post-stress (Fig. 2C).

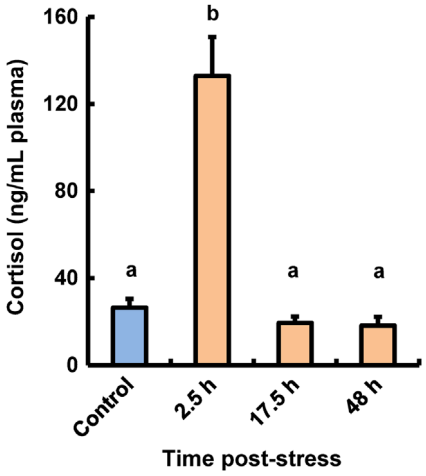

Fig. 1. Effect of thermal stressors on cortisol levels in plasma of coho salmon *O. kisutch*. Data represent means ± SEM ($n=8$). Statistical relationships between groups are indicated by letters where significant differences were detected ($p<0.05$).