Aquaculture 318 (2011) 379–383

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aqua-online

Antioxidant enzyme activities in Pacific white shrimp (*Litopenaeus vannamei*) in response to environmental hypoxia and reoxygenation

Delia Patricia Parrilla-Taylor, Tania Zenteno-Savín *

Planeación Ambiental y Conservación, Centro de Investigaciones Biológicas del Noroeste, S.C., La Paz, Baja California Sur, Mexico

ARTICLE INFO

Article history:
Received 23 December 2010
Received in revised form 18 March 2011
Accepted 5 May 2011
Available online 13 May 2011

Keywords:
Antioxidant enzymes
Hypoxia
Reoxygenation
White shrimp

ABSTRACT

Hypoxia–reoxygenation represents a relevant physiological challenge for shrimp culture. The sudden input of oxygen (O_2) after environmental hypoxia increases reactive oxygen species (ROS) production in Pacific white shrimp (*Litopenaeus vannamei*). It has been postulated that a relatively high activity of the main antioxidant enzymes allows several adapted invertebrate species to survive repetitive cycles of hypoxia–reoxygenation by counteracting ROS production and, thus, preventing oxidative stress. In this study, we evaluated the activity of the antioxidant enzymes superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) in extracts of muscle and hepatopancreas from white shrimp exposed to short-term (24 h) hypoxic conditions (1 mg L^{-1} of dissolved O_2), and subsequent reoxygenation cycles (1 to 5 h, in 1 h intervals). Results show differences in antioxidant enzyme activities among tissues, even under control conditions. SOD activity was higher in muscle than in hepatopancreas whereas CAT and GPx activities were higher in hepatopancreas. In muscle, SOD activity was higher during hypoxia and lower after 1 h of reoxygenation. In hepatopancreas, antioxidant enzyme activities were higher during hypoxia and lowest in the first hour of reoxygenation. Significant changes in SOD, CAT and GPx activities were also observed in both tissues after 1 h and 2 h of reoxygenation suggesting that hypoxia–reoxygenation affects the antioxidant enzymes. These changes in antioxidant enzyme activities in response to hypoxia–reoxygenation may be crucial to avoid oxidative damage and to preserve the quality of the aquaculture product.

1. Introduction

Oxygen (O_2) concentration is the major variable limiting water quality for the intensification of shrimp aquaculture. The constant exposure of cultured shrimp to low environmental O_2 concentrations (hypoxia) and subsequent increases in dissolved O_2 content (reoxygenation) affects the behavior and physiology of crustaceans (Chen et al., 2001; Cheng et al., 2003). Hypoxic conditions cause stress and alter the optimal development of shrimp resulting in a reduced frequency of molts, slow growth and death (Allan and Maguire, 1991; Boyd and Watten, 1989; Herreid, 1980; Hopkins et al., 1993; Wu, 2002).

During respiration, approximately 0.1–0.2% of the oxygen consumed by aerobic cells is converted to reactive oxygen species (ROS) (Fridovich, 2004). ROS are highly reactive molecules that can oxidize cellular components leading to the development of many pathological conditions (Halliwell and Gutteridge, 2001). Endogenous antioxidant defenses are crucial for the control of ROS production and the prevention of oxidative damage (Lesser, 2006). Under hypoxic conditions, accumulated electrons are available for ROS formation using whatever oxygen remains, resulting in the generation of high levels of ROS (Storey, 1996). After hypoxia, the sudden increase of O_2 (reoxygenation) restores energy potential but also reacts with accumulated electrons in mitochondria, via one-electron mechanism, and with accumulated degradation products, such as hypoxanthine and xanthine, resulting in an increased ROS production and, thus, oxidative stress (Halliwell and Gutteridge, 2001; Li and Jackson, 2002; Ranby and Rabek, 1978).

The endogenous antioxidant system counteracts the effects of ROS and plays a crucial role in protecting cells from oxidative stress. This system is composed of enzymes and other (non-enzyme) molecules that scavenge ROS. The antioxidant enzymes are the first line of defense against ROS and include superoxide dismutase (SOD), which converts superoxide radical ($O_2^{\bullet -}$) to peroxide (H_2O_2); catalase, which reduces H_2O_2 to water, and glutathione peroxidase (GPx), which detoxifies H_2O_2 and organic hydroperoxides (Di Giulio et al., 1995; Halliwell and Gutteridge, 2001).

The evaluation of the antioxidant system has been consistently used as a potential indicator of oxidative stress in several marine invertebrates naturally exposed to hypoxia–reoxygenation (Abele and Puntarulo, 2004; Guerriero et al., 2002; Martínez-Alvarez et al., 2005; Pannunzio and Storey, 1998). Even so, only a few studies on the

* Corresponding author at: Planeación Ambiental y Conservación, Centro de Investigaciones Biológicas del Noroeste, S.C., Mar Bermejo 195, Col. Playa Palo de Santa Rita, La Paz, Baja California Sur 23090, México. Tel.: +52 612 123 8502; fax: +52 612 125 3625.
E-mail address: tzenteno04@cibnor.mx (T. Zenteno-Savín).

0044-8486/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.aquaculture.2011.05.015

effects of hypoxia–reoxygenation in crustaceans have been published to date (de Oliveira et al., 2005; Zenteno-Savín et al., 2006; García-Triana et al., 2010). Hypoxia–reoxygenation increases ROS production in cultured Pacific white shrimp without increasing oxidative damage (Zenteno-Savín et al., 2006). The effects of hypoxia–reoxygenation on the antioxidant enzyme system in cultured shrimp have not been fully investigated. The aim of this work was to analyze the activity of the antioxidant enzymes SOD, CAT and GPx in Pacific white shrimp exposed to hypoxia and reoxygenation in order to contribute to the elucidation of the mechanisms that allow them to counteract ROS production and to prevent oxidative damage. We hypothesized that reoxygenation after environmental hypoxia increases antioxidant enzyme activities in cultured Pacific white shrimp.

2. Methods

2.1. Experimental organisms

Juvenile Pacific white shrimp (10–15 g body mass) were obtained from supra-littoral pools at Centro de Investigaciones Biológicas del Noroeste (CIBNOR), La Paz, Mexico. Animals were acclimatized for a week in 1500 L aquaria (50 shrimp per m^3) under controlled conditions (24.9 ± 0.1 °C, 6.0 ± 0.1 mg O_2 L^{-1}, 37.8 ± 0.1 ppt salinity, 12 h/12 h dark/light cycles). Only shrimp in the inter-molt stage were used. Molt stage was determined by the examination of uropoda (Robertson et al., 1987). Shrimp were fed twice daily with a specially-formulated shrimp diet (CIBNOR). After the acclimatization period, animals were transferred to experimental aquaria (4 L capacity) supplied with air stones and held at 24.9 ± 0.1 °C, 6.0 ± 0.1 mg O_2 L^{-1}, 37.8 ± 0.1 ppt salinity for 24 h. Shrimp were then divided into three groups. Control group ($n = 6$) was maintained under normoxic conditions (6.0 ± 0.1 mg O_2 L^{-1}) for 24 h. Hypoxia group ($n = 6$) was exposed to 1.0 ± 0.05 mg O_2 L^{-1} for 24 h; oxygen levels in this group were decreased by bubbling with N_2 gas until the desired O_2 concentrations were reached; oxygen levels were maintained by adding N_2 gas when needed. Reoxygenation group was exposed to 24 h hypoxia as described earlier, followed by a constant supply of air until the tanks reached normoxic conditions (6.0 ± 0.1 mg O_2 L^{-1}); reoxygenation was maintained for 1, 2, 3, 4 and 5 h ($n = 6$ in each group). At the end of the experiments, shrimp were sacrificed and immediately frozen by immersion in liquid nitrogen. Hepatopancreas and muscle tissues from each shrimp were excised and kept frozen at −80 °C until analyzed. Separate measurements were made on each individual shrimp.

2.2. Biochemical assays

2.2.1. Tissue homogenates

Each sample (50 mg, wet tissue) was homogenized in two volumes of potassium phosphate buffer (0.1 M, pH 7.5) containing 60 mM EDTA and 1 mM PMSF for 2 min. Homogenates were centrifuged at 3000 ×*g* for 15 min at 4 °C. Supernatants were removed and immediately used for antioxidant enzyme activity analyses, which were carried out using a spectrophotometer (Jenway 6305 uv/vis, Jenway Ltd., London, U.K.). Assays were run in triplicate. Total protein concentration in tissue homogenates was measured by using the Biorad® protein assay kit.

2.2.2. Superoxide dismutase (E.C. 1.15.1.1)

Total superoxide dismutase (SOD) activity was measured by following the method of Suzuki (2000), which uses xanthine/xanthine oxidase as a $O_2^{\bullet -}$ generator, and nitro blue tetrazolium (NBT) as a detector. Each sample was diluted 1:20 with potassium phosphate buffer (0.1 M, pH 7.5, 60 mM EDTA). Sodium carbonate working solution (50 mM, 0.1 mM xanthine, 0.025 mM NBT, 0.1 mM EDTA), xanthine oxidase (0.1 μM mL-1 in 2 M ammonium sulfate), and sample or blank (potassium phosphate buffer) were mixed in a cuvette. The change in absorbance per minute at 560 nm was calculated. Enzymatic activity was expressed in U mg^{-1} protein. One unit of SOD activity is defined as the amount of enzyme needed to inhibit the reaction of $O_2^{\bullet -}$ with NBT by 50%.

2.2.3. Catalase (E.C. 1.11.1.6)

Catalase (CAT) activity was measured according to Aebi (1984) by following the decrease in absorbance at 240 nm due to H_2O_2 consumption. The reaction mixture contained 100 mM potassium phosphate buffer and 10 mM H_2O_2. The change in absorbance per minute was calculated. Enzyme activity was expressed in U mg^{-1} protein. One unit of CAT is defined as the amount of enzyme needed to reduce 1 μmol of H_2O_2 min^{-1}.

2.2.4. Glutathione peroxidase (E.C. 1.11.1.9)

Selenium-dependent glutathione peroxidase (GPx) activity was monitored according to the method described by Folhé and Gunzler (1984), by following the decrease of NADPH at 340 nm using H_2O_2 as substrate. In a cuvette, 500 mM potasium phosphate buffer (pH 7.2), 50 mM EDTA, 20 mM sodium azide, 15 U mL^{-1} glutathione reductase (GR), 1.5 mM NADPH, 250 mM glutathione (GSH), sample, and H_2O_2 were mixed. The change in absorbance was recorded every 40 s for 3 min. Enzyme activity was expressed in U mg^{-1} protein. One unit of activity is defined as the amount of enzyme needed to oxidize 1 mmol of GSH in 1 min.

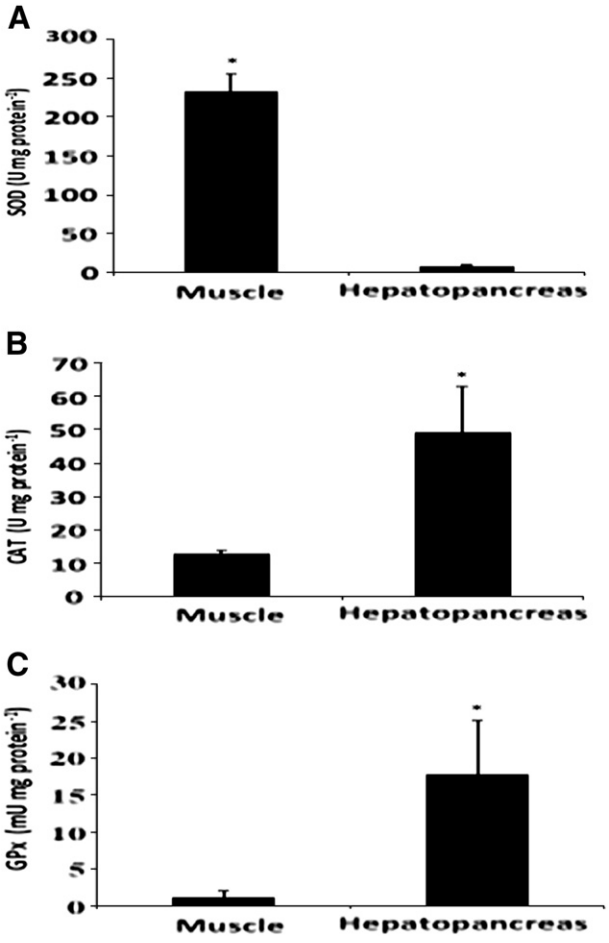

Fig. 1. (A) Superoxide dismutase (SOD, U mg of protein^{-1}), (B), catalase (CAT, U mg of protein^{-1}) and (C), glutathione peroxidase (GPx, mU mg of protein^{-1}) activity in hepatopancreas and muscle from pacific white shrimp (*Litopenaeus vannamei*) under control condition. Results are expressed as mean ± SEM. * = significant differences ($p < 0.05$).