Chemosphere 82 (2011) 377–389

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

Interactive effects of cadmium and hypoxia on metabolic responses and bacterial loads of eastern oysters *Crassostrea virginica* Gmelin

Anna V. Ivanina [a], Brett Froelich [a], Tiffany Williams [a], Eugene P. Sokolov [b], James D. Oliver [a], Inna M. Sokolova [a,*]

[a] Biology Department, University of North Carolina at Charlotte, 9201 University City Blvd., Charlotte, NC 28223, United States
[b] Department of General Surgery, Carolinas Medical Center, 1000 Blythe Blvd., Charlotte, NC 28203-5871, United States

ARTICLE INFO

Article history:
Received 30 June 2010
Received in revised form 20 September 2010
Accepted 26 September 2010
Available online 23 October 2010

Keywords:
Bivalves
Aerobic metabolism
Glycolysis
Energy status
Transcript expression
Vibrio spp.

ABSTRACT

Pollution by toxic metals including cadmium (Cd) and hypoxia are important stressors in estuaries and coastal waters which may interactively affect sessile benthic organisms, such as oysters. We studied metabolic responses to prolonged hypoxic acclimation (2 weeks at 5% O_2) in control and Cd-exposed (30 d at 50 μg L^{-1} Cd) oysters *Crassostrea virginica,* and analyzed the effects of these stressors on abundance of *Vibrio* spp. in oysters. Hypoxia-acclimated oysters retained normal standard metabolic rates (SMR) at 5% O_2, in contrast to a decline of SMR observed during acute hypoxia. However, oysters spent more time actively ventilating in hypoxia than normoxia resulting in enhanced Cd uptake and 2.7-fold higher tissue Cd burdens in hypoxia. Cd exposure led to a significant decrease in tissue glycogen stores, increase in free glucose levels and elevated activity of glycolytic enzymes (hexokinase and aldolase) indicating a greater dependence on carbohydrate catabolism. A compensatory increase in activities of two key mitochondrial enzymes (citrate synthase and cytochrome c oxidase) was found during prolonged hypoxia in control oysters but suppressed in Cd-exposed ones. Cd exposure also resulted in a significant increase in abundance of *Vibrio parahaemolyticus* and *Vibrio vulnificus* levels during normoxia and hypoxia, respectively. Overall, Cd- and hypoxia-induced changes in metabolic profile, Cd accumulation and bacterial flora of oysters indicate that these stressors can synergistically impact energy homeostasis, performance and survival of oysters in polluted estuaries and have significant consequences for transfer of Cd and bacterial pathogens to the higher levels of the food chain.

1. Introduction

Anthropogenic pollution and oxygen depletion are serious environmental problems in marine habitats worldwide. Reduced oxygen levels (hypoxia) or complete lack of oxygen (anoxia) typically occur in the near-bottom waters of the coastal zones and estuaries and are often associated with anthropogenic eutrophication fueling algal blooms, microbial respiration and oxygen depletion (Diaz and Rosenberg, 2008). In severe cases, such oxygen depletion may result in formation of the "dead zones" characterized by mass mortalities of benthic organisms. However, even moderate hypoxia may result in significant physiological and behavioral disturbances of the resident biota and lead to negative consequences for their growth, reproduction and long-term population survival (Baker and Mann, 1992; Diaz and Solow, 1999; Rabalais et al., 1999).

Periodical oxygen deprivation in estuaries and coastal waters often co-occurs with other stressors such as metal pollutants that can interactively affect physiology of benthic organisms. A trace metal cadmium (Cd) is an important persistent pollutant that is highly toxic, widely distributed in the environments and can adversely affect organisms at relatively low concentrations (Strydom et al., 2006). As a major water pollutant, it can accumulate in marine organisms and induce detrimental physiological effects such as metabolic or osmoregulatory dysfunction (Lionetto et al., 1998, 2000; Sokolova and Lannig, 2008). One of the key aspects of Cd toxicity is its strong inhibitory effect on aerobic metabolism and mitochondrial function that results in cellular energy deficiency and oxidative stress (Stohs and Bagchi, 1995; Sokolova, 2004; Lannig et al., 2006a, 2008; Cherkasov et al., 2007). Cd exposure can also suppress anaerobic metabolism in facultative anaerobes such as intertidal bivalves and impair their ability to recover after anoxic stress (Strydom et al., 2006; Kurochkin et al., 2009). The strong impact of Cd on energy metabolism makes it a prime candidate for interference with other stress tolerance mechanisms that critically

* Corresponding author. Tel.: +1 704 687 8532; fax: +1 704 687 3128.
E-mail address: isokolov@uncc.edu (I.M. Sokolova).

0045-6535/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.chemosphere.2010.09.075

depend on metabolic regulation and maintenance of energy homeostasis. Indeed, synergistic effects of Cd and temperature stress has been shown in a variety of aquatic ectotherms where Cd exposure led to a reduction in thermal tolerance and *vice versa* (review in: Sokolova and Lannig, 2008). In contrast, the interactive effects of Cd exposure and hypoxia on marine organisms are not well understood. Given the widespread coastal pollution and an alarming rate of increase of the coastal dead zones (5.54% year^{-1}) (Vaquer-Sunyer and Duarte, 2008), more research is urgently needed to understand the interactive impacts of hypoxia and metal pollutants on benthic organisms.

Eastern oysters *Crassostrea virginica* are ecologically and economically important species serving as ecosystem engineers in western Atlantic estuaries. Oysters are exposed to Cd via the contact with water, sediment and contaminated food (predominantly algae) in polluted estuaries and can accumulate high levels of Cd in their bodies making them susceptible to the toxic effects of this metal (Roesijadi, 1996; O'Connor and Lauenstein, 2006). Oysters play a key role in the dynamics of Cd in estuarine ecosystems serving as major vectors for accumulation and trophic transfer of this metal (Pigeot et al., 2006). They also serve as vectors for several important bacterial pathogens of humans and other vertebrates including highly pathogenic *Vibrio* spp. such as *Vibrio cholerae, Vibrio vulnificus*, *Vibrio parahaemolyticus* and *Vibrio alginolyticus* (Thompson and Swings, 2006). Like other marine intertidal bivalves, oysters are among the animal champions of hypoxia tolerance. They are routinely exposed to oxygen-deficient conditions during air exposure at low tide as well as to prolonged periods of hypoxia or anoxia that can last from several days to several weeks in estuarine and coastal "dead zones" (Diaz and Solow, 1999; Greenway and Storey, 1999; Lenihan et al., 2001). The mechanisms responsible for a short-term endurance of hypoxia and anoxia are well studied in intertidal mollusks and involve reversible metabolic arrest, maintenance of the large reserves of fermentable fuels such as glycogen or aspartate, and alternative pathways of fermentative metabolism that increase ATP yield per unit metabolized substrate (Larade and Storey, 2002; David et al., 2005). In contrast, little is known about the potential effects of prolonged hypoxia and Cd exposure on oyster physiology and metabolism.

The goals of this study were to determine the interactive effects of prolonged hypoxia and Cd exposure on aerobic and anaerobic metabolism and tissue energy status (including levels of adenylates, free glucose and glycogen reserves) of eastern oysters *C. virginica*. We also determined mRNA expression and activity of key glycolytic and mitochondrial enzymes (hexokinase, aldolase, citrate synthase and cytochrome c oxidase) as well as mRNA expression of key regulatory genes involved in O_2 sensing and homeostasis (the hypoxia-inducible factor 1α or HIF1-α, and prolyl hydroxylase 2, or PHD2) in order to gain insight into the molecular mechanisms of metabolic responses of oysters to combined Cd and hypoxia stress. For hypoxic exposures, we selected 5% O_2 which is within the range of concentration found in the coastal "dead zones" (Baker and Mann, 1992; Diaz and Solow, 1999; Rabalais et al., 1999), is close to the critical partial oxygen pressure (P_cO_2) leading to metabolic rate depression during acute exposures but is not lethal to oysters (see "Results" below). For Cd exposures, we used 50 μg L^{-1} waterborne Cd (as $CdCl_2$); this concentration is within the range of Cd levels found in polluted estuaries (15–80 μg L^{-1} Cd; GESAMP, 1987; Crompton, 1997; Hackney et al., 1998). Our previous studies showed that this concentration of Cd elicits significant physiological response (but no acute toxicity) in oysters and results in physiologically relevant tissue Cd burdens similar to those found in oysters from polluted estuaries (Sokolova et al., 2005; Cherkasov et al., 2006a and references therein). Thus, this study provides insights into the effects of long-term exposures to sublethal, environmentally relevant levels of Cd and hypoxia stress and their combination in oysters. Understanding the impacts of Cd and hypoxia on energy metabolism is critical for assessing the organismal and population-level consequences of these stressors because stress-induced disturbances of energy homeostasis have direct consequences for the organism's fitness (Calow, 1989, 1991; Sibly and Calow, 2009). We also quantified tissue loads of commensal *Vibrio* spp. in oysters (focusing on human pathogens *V. cholerae*, *V. vulnificus*, *V. parahaemolyticus* and *V. alginolyticus*) in order to test the hypothesis that prolonged exposure to hypoxia, Cd or their combination increases the abundance of these facultatively anaerobic bacteria and thus can affect the dynamics of these pathogens in polluted estuaries.

2. Materials and methods

2.1. Chemicals

Unless otherwise indicated, all chemicals and enzymes were purchased from Sigma Aldrich (St. Louis, MO, USA), Roche (Indianapolis, IN, USA) or Fisher Scientific (Pittsburg, PA, USA) and were of analytical grade or higher.

2.2. Animal collection and maintenance

Oysters (*C. virginica*) were purchased from commercial oyster suppliers (Cuttyhunk Shellfish Farms, Cuttyhank, MA and Taylor Shellfish Farms, Shelton, WA), shipped overnight to the University of North Carolina at Charlotte and placed in recirculated aerated tanks with artificial seawater (Instant Ocean®, Kent Marine, Acworth, USA) at 20 ± 1 °C and 30 ± 1‰. Oysters were allowed to recover for 10 d. After this preliminary acclimation, half of the tanks were randomly selected, and Cd (as $CdCl_2$) was added to the nominal concentration of 50 μg L^{-1}. The remaining tanks were used as controls. To avoid pseudoreplication, at least three tanks were set for control or Cd exposure, and oysters were randomly sampled from these tanks for each experiment. Oysters were acclimated in tanks with or without Cd addition for 30 d prior to hypoxic exposures. Mortality during this acclimation period was less than 5% and did not significantly differ between control and Cd-exposed oysters.

After 30 d of acclimation, control and Cd-exposed oysters were randomly divided into four groups and exposed for 2 weeks to either normoxia or hypoxia. Cd concentrations in the water were maintained at the same levels as during the preceding 30-d acclimation period (i.e. control oysters were exposed to clean ASW and Cd-exposed oysters were kept in ASW with 50 μg L^{-1} Cd). This resulted in the following four experimental groups: (1) normoxia (21% O_2 or 100% air saturation) with no Cd addition (normoxic controls); (2) normoxia (21% O_2) exposed to 50 μg L^{-1} Cd (normoxic Cd-exposed oysters); (3) hypoxia (5% O_2 or 24% air saturation) with no Cd addition (hypoxic controls); (4) hypoxia (5% O_2) exposed to 50 μg L^{-1} Cd (hypoxic Cd-exposed oysters). For each experimental group, two replicate trays were set up, each containing 10–12 oysters in 5 L ASW. In normoxic treatments, water was aerated with ambient air. Hypoxia was achieved by bubbling ASW with a certified gas mixture containing 5% O_2, 0.04% CO_2 and balance of nitrogen (Roberts Oxygen, Charlotte, NC). Gas content of the mixtures was analyzed by the manufacturer and certified to be accurate within 10% of the target values (Roberts Oxygen, Charlotte, NC). Oxygen concentrations were tested periodically throughout the experimental exposures and were 97–100% and 20–30% of air saturation (corresponding to 7.3–7.5 and 1.5–2.3 mg O_2 L^{-1}, respectively) in normoxia and hypoxia, respectively. Water in each tray was changed every other day using clean or Cd-spiked (50 μg L^{-1}