Journal of Trace Elements in Medicine and Biology 34 (2016) 70–78

Contents lists available at ScienceDirect

Journal of Trace Elements in Medicine and Biology

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

Investigation of thermostable metalloproteins in *Perna perna* mussels from differentially contaminated areas in Southeastern Brazil by bioanalytical techniques

Raquel Teixeira Lavradas, Rafael Christian Chávez Rocha, Tatiana Dillenburg Saint' Pierre, José Marcus Godoy, Rachel Ann Hauser-Davis*

Pontifícia Universidade Católica—Rio de Janeiro (PUC-Rio), Departamento de Química, Rua Marquês de São Vicente, 225, Gávea, Rio de Janeiro, RJ CEP: 22453-900, Brazil

ARTICLE INFO

Article history:
Received 4 September 2015
Received in revised form
25 November 2015
Accepted 4 January 2016

Keywords:
Metalloproteins
Metals
Mussels
SDS-PAGE
HPLC-SEC-ICP-MS

ABSTRACT

Metallomic studies regarding environmental contamination by metals are of value in elucidating metal uptake, trafficking, accumulation and metabolism in biological systems. Many proven bioindicator species, such as bivalves, have not yet, however, been well-characterized regarding their metalloprotein expression in response to environmental contaminants. In this context, the aim of the present study was to investigate metalloprotein expressions in the thermostable protein fraction of muscle tissue and digestive glands from mussels (*Perna perna*) from three differentially metal-contaminated sites in Southeastern Brazil in comparison with a reference site. The thermostable protein fractions were analyzed by SDS-PAGE and SEC-HPLC-ICP-MS. Metal content was also determined in both the crude and the purified extracts. Several inter-organ differences were observed, which is to be expected, while inter-site differences regarding thermostable protein content were also verified, indicating accumulation of these elements in muscle tissue and digestive glands and disruption of homeostasis of essential elements, with detoxification attempts by metal-bound proteins, since all metalloproteins present in both matrices eluted bound to at least one non-essential metal. These results are also noteworthy with regard to the adopted reference site, that also seems to be contaminated by toxic metals.

1. Introduction

The recently developed field of metallomics investigates the metal and metalloid species present in a cell or tissue according to their identity, quantity and localization. This area of interest includes the study of metal-bound proteins, or metalloproteins, focusing on their structural and functional characterization, identification and quantification in living organisms [1–4]. This field of knowledge is interdisciplinary, combining analytical, inorganic and biochemical studies, with an ultimate goal to elucidate metal uptake, trafficking, accumulation and metabolism in biological systems [5,6].

Several metalloproteins are currently being applied as successful biomarkers for environmental contamination, including metal exposure, in different organisms [7,8]. The interest in monitoring the responses to metal exposure in living organisms has increased in the last years, since they can reflect the effect of these pollutants on cellular metabolism, trafficking and global homeostasis [7]. In this context, systematic studies on metal uptake, trafficking, and function in many basic and complex biological processes aid in the understanding of the molecular mechanisms underlying beneficial or toxic effects exerted by metals or metalloids, and further clarify their impact on living organisms [9].

Mussel farming is an expressive economic activity in Brazil, and these organisms are consumed by a great part of the population in practically all coastal zones in the country. The mussel *Perna perna* is one of the most commercially cultivated bivalves in Brazil [10], with a production in the order of 12.500 t in the last decades, representing 19% of the total produced by the entire Brazilian mariculture [11]. This organism is considered an adequate sentinel species for environmental contamination, including metal exposure, since it is sessile and a filter-feeder [12–15]. Many studies regarding metal exposure have been conducted with *Perna perna* mussels and some other species from this genus, including the investigation of different proteomic and enzymatic biomarkers [16–19]. Since, however, metalloproteins are often of no partic-

* Corresponding author.
E-mail address: rachel.hauser.davis@gmail.com (R.A. Hauser-Davis).

http://dx.doi.org/10.1016/j.jtemb.2016.01.003
0946-672X/

ular interest in conventional proteomics studies [20], and the thermostable protein fraction even less so, the thermostable metalloprotein expression of this species, and, in fact, of bivalves in general, in response to environmental contaminants has not been well-characterized.

In this context, the present study reports comparative proteomic analyses, metal determinations and metallomic investigations applying the SEC-HPLC-ICP-MS technique to investigate changes in the metalloprotein expression of essential (Cu, Zn, Ni, Se) and non-essential (Hg, Pb, Cd) elements in the muscle tissue and digestive glands mussels, both in the crude matrix and the thermostable metalloprotein fraction of each organ, from four differentially metal-contaminated sites in Rio de Janeiro, Brazil.

2. Material and methods

2.1. Study area

Guanabara Bay (GB) is one of the largest and most important estuaries of the Brazilian coast. It is located in the state of Rio de Janeiro, is composed of an area of approximately 400 km^2, and is surrounded by 11 million inhabitants. Some areas of the bay receive environmental impacts on a daily basis, such as domestic sewage and non-treated industrial effluents, both domestic and from the 12,000 industries, oil refineries, two navy bases and shipyards around the bay. Because of this, certain areas of the bay suffer the effects of organic matter, oils, organic compounds and heavy metals [21]. Even with all these impacts, however, the bay is still a significant fishing site of social and economic importance, retaining about 90 km^2 of fringing mangroves [22], which maintain a high local biota diversity.

Ilha Grande Bay (IG), located on the southern coast of the state of Rio de Janeiro (city of Angra dos Reis) is a very rugged bay, composed of several smaller bays, such as Paraty and Ribeira bays and Frade Cove, and dotted with 365 islands, which decreases the hydrodynamics of the area. It is an important region for the tourist market of Rio de Janeiro and considered one of the most important areas of this state in terms of fishing productivity [23,24]. Ilha Grande Bay is also sheltered from anthropogenic impacts and has been considered a reference area for certain contaminants, such as metals, over the years [25,26].

These study areas are displayed in Fig. 1, modified from Seixas et al. [27].

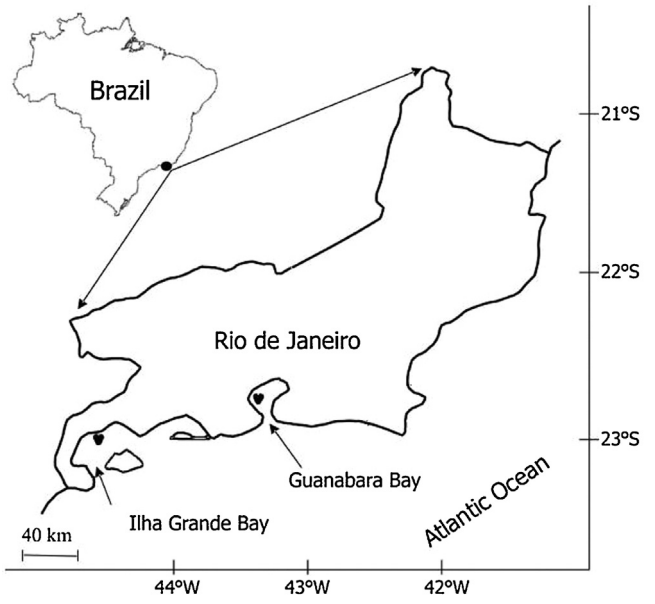

Fig. 1. Map of the study areas Ilha Grande Bay and Guanabara Bay, modified from Seixas et al. [27].

2.2. Sampling

Perna perna individuals were sampled from 4 different beaches located in Southeastern Brazil: 3 belonging to the impacted Guanabara Bay, namely Diabo beach (DB; $N = 40$), Vermelha Beach (VB; $N = 40$) and Urca Beach (UB; $N = 40$), and a beach from the reference site, Ilha Grande Bay (IG; $N = 40$). Individuals were measured (shell length, width and height) and grouped according to size, totaling 4 composite samples from each beach, each containing 10 individuals. Muscle tissue and digestive glands were separated, pooled, frozen at −20 °C in sterile polypropylene tubes and freeze-dried (Liotop 101, Liobrás, SP, Brazil) for 48 h.

2.3. Sample processing

The muscle and digestive gland pools were processed for protein extraction according to the protocol by Erk et al., with some modifications, by thermal extraction [28] using TCEP as a reducing agent [29]. This thermostable fraction is expected to show lower protein content than crude extracts, due to the denaturing of most proteins at temperatures above 60–70 °C, and consequently, is a less complex matrix with several advantages, such as being easier to analyze by certain analytical techniques. Briefly, the samples were homogenized at a 1:20 ratio in a buffer solution containing Tris-HCl, 2.0×10^{-2} mol L^{-1} pH 8.6, PMFS 5.0×10^{-4} mol L^{-1} and TCEP 0.01% and centrifuged at 20,000 × *g* for 60 min at 4 °C. The supernatants were then carefully removed and transferred to other tubes, heated at 70 °C for 10 min in a thermostatic water bath and centrifuged again at 20,000 × *g* for 30 min at 4 °C. After this last step, the supernatants were again separated and the samples were frozen at −20 °C prior to subsequent analyses.

2.4. General protein content of the purified thermostable fraction by SDS-PAGE

SDS-PAGE analyses were carried out in order to qualitatively investigate and compare the protein content of the purified thermostable fraction of the muscle tissue and digestive glands from the *Perna perna* mussel samples. Total protein content was quantified by the Lowry method modified by Peterson using Bovine serum Albumin (BSA) as standard [30], in order to apply the same amount of protein to each gel lane (20 μg). Protein separations were carried out on 15% sodium dodecyl sulfate polyacrylamide gels (SDS-PAGE). Gels were stained by silver staining as described previously [31]. The molecular weights of the protein bands observed on the gels after staining were determined using Biorad's Precision Plus Protein™ Dual Color Standards. The gels were scanned on an ImageScanner II (GE Healthcare, Uppsala, Sweden) with the densitometer operating at 300 dpi. The Image-Master 2D Platinum 6.0 software package (GeneBio, Geneva, Switzerland) was employed for the gel imaging analyses.

2.5. Characterization of the metalloprotein expression of the purified thermostable fractions by SEC-HPLC-ICP-MS

Chromatographic separations were performed using a Model 1100 HPLC pump with a UV detector (Shimadzu, São Paulo, Brazil) as the delivery system. Elemental detection was performed using a NexIon 300X quadrupole inductively coupled plasma mass spectrometer (PerkinElmer, Norwalk, CT, USA) equipped with a collision and reaction cell. The ICP-MS measurement conditions were