Hydrothermal Vents as a Possible Natural Pollution Laboratory: Metal Detoxification Mechanisms in Mussels and Fishes from Lucky Strike

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ABSTRACT

Deep-sea hydrothermal vent environments are considered highly toxic with high temperatures, sulphide, methane, carbon dioxide and heavy metals. Paradoxically, life at the vents exhibits high productivity and therefore provides a unique way to study the effect of long-term adaptation to environmental stress. The aim of this study was to compare the responses of some biomarkers of metal exposure between two hydrothermal species, the mussel Bathymodiolus azoricus and the chimera fish Hydrolagus pallidus from Lucky Strike vent field. Results show high metal levels in B. azoricus compared to their coastal counterparts, especially for Ag, Cd, Cu and Zn in the gills. However, metallothionein (MT) levels were surprisingly low taking into account the metal accumulation. H. pallidus also exhibited significantly higher metal concentrations in the gills than the muscle, however with MT levels 2-fold higher in the muscle. Superoxide dismutase (SOD) and catalase (CAT) activities in the mussels were significantly higher in the gills, while glutathione peroxidases (GPx) were present mainly in the mantle, suggesting the two tissues have different antioxidant responses to deal with stress factors. Lipid peroxidation (LPO) was more evident in the gills, indicating that defence mechanisms in this tissue may not be completely effective against metal toxic-ity. Although the major food source for H. pallidus consists in metal overloaded mussels, no evidences of metal bioamplification were found. Chimaeras may possess other detoxification mechanisms that can be more important than MT.

Key words: Bathymodiolus azoricus, Hydrolagus pallidus, metallothioneins, antioxidant enzymes, lipid peroxidation.

RESUMO

Fontes hidrotermais como possíveis laboratórios naturais de poluição: mecanismos de destoxificação de metais em mexilhões e peixes de Lucky Strike

As fontes hidrotermais são consideradas ambientes extremamente tóxicos, caracterizadas por elevadas temperaturas e presença de grandes concentrações de sulfuretos, metano, dióxido de carbono e metais. Paradoxalmente, a vida nas fontes hidrotermais apresenta grande produtividade e por isso elas constituem um meio único para estudar os efeitos adaptativos a longo prazo ao stress ambiental. O objetivo deste estudo foi comparar as respostas de alguns biomarcadores de exposição metálica en-tre duas espécies hidrotermais, o mexilhão Bathymodiolus azoricus e o peixe quimera Hydrolagus pallidus do campo hidrotermal Lucky Strike. Os resultados mostraram que o mexilhão B. azoricus apresenta maiores valores de metais quando comparados com espécies coasteiras aparentadas, especialmente para Ag, Cd, Cu e Zn nas brânquias. No entanto, os níveis de metalotionina (MT) foram surpreendentemente baixo tendo em conta a acumulação metálica observada. A quimera H. pallidus também apresentou níveis de metais significativamente superiores nas brânquias em comparação ao músculo, no entanto, os valores de MT foram duas vezes superiores no músculo. A atividade das enzimas superóxido dismutase (SOD) e catalase (CAT) nos mexilhões foi significativamente superior nas brânquias, enquanto as glutathiones peroxidases (GPx) foram mais importantes no manto, o que sugere que os dois tecidos apresentam diferentes estratégias antioxidantes para lidar com fatores de stress. A peroxidação lipídica (LPO) foi mais evidente nas brânquias, indicando que os mecanismos de defesa nesse tecido podem não ser completamente eficazes contra a toxicidade metálica. Apesar de a principal fonte de alimento de H. pallidus serem mexilhões com elevados níveis de metais, não se encontraram evidências de bioamplificação de metais. As quimeras poderão apresentar outros mecanismos de desintoxicação mais importantes que as MT.

Palavras-chave: Bathymodiolus azoricus, Hydrolagus pallidus, metallothioneins, enzimas antioxidantes, peroxidação lipídica.

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INTRODUCTION

Hydrothermal vents were discovered in 1977 and since then researches was conducted to understand how organisms could survive in a very extreme environment, near vent chimneys that emanate hot, acidic and anoxic fluids, extremely enriched with metals and hydrogen sulphide that would normally be toxic in such amounts and combinations (Sarradin et al., 1998). In this context, hydrothermal vents were lately thought that could be used as a possible natural pollution laboratory (Prusky & Dixon, 2002). Bathymodiolus azoricus is one of the most abundant species in Mid-Atlantic Ridge vents (Desbruyères et al., 2000) and Hydrologus pallidus is a deep-sea fish found near hydrothermal environments that feeds mainly on vent mussels (Marques & Porteiro, 2000).

Metals are known to produce deleterious effects in marine organisms at high concentrations, including the production of reactive oxygen species responsible for oxidative cellular damage (Stoks & Bagchi, 1995). Organisms developed several detoxification mechanisms to deal with metal contamination including the synthesis of metallothioneins (MT) and antioxidant enzymes. MT is a low-molecular-weight protein with high sulphhydryl content and high affinity for groups IB and IIB metal ions, thereby playing an essential role in cellular processes of metal handling and detoxification. These proteins are induced by heavy metals but also by different chemicals and stressors, in particular by oxidants (Bauman et al., 1991). Antioxidant enzymes include the superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidases (GPx) (Fridovich, 1978). Lipid peroxidation (LPO) is an important feature in cellular injury, resulting from free radical reactions in biological membranes (Chessem, 1982). Transition metals might stimulate LPO (Halliwell & Gutteridge, 1984).

The aim of this study was to determine the concentrations of Ag, Cu, Cd, Fe, Mn and Zn in B. azoricus and H. pallidus from Lucky Strike and some detoxification mechanisms such as metallothioneins (MT) in the gills and mantle of mussels and the gills and muscle of chimaeras. Antioxidant enzymes and lipid peroxidation (LPO) were only studied in mussels.

MATERIAL AND METHODS

Sample collection and preservation

Both mussels (Bathymodiolus azoricus, n = 10) and fishes (Hydrologus pallidus, n = 6) were collected in the Lucky Strike hydrothermal vent area (37°17′N – 32°16′W; 1580-1711 m deep) during the Ventox project in summer 2001. Mussels were collected using a remote operated vehicle during the ATOS cruise on board of the research vessel Atalante (IFREMER – French Research Institute for Exploitation of the Sea). Living organisms were immediately dissected and the gills and mantle were deep frozen in liquid nitrogen until analysis. Fishes were captured using baited bottom long-line during VENTACO cruise on board of the research vessel Arquipelago (Department of Oceanography and Fisheries – University of Azores). The fishes were already dead on arrival, which unable to determine antioxidant enzyme activities due to their fast degradation, and the gills and muscle were dissected and frozen in liquid nitrogen until analysis.

Metal analysis

The concentration of Ag, Cd, Cu, Fe, Mn and Zn were determined in both tissues of B. azoricus and H. pallidus by ETAAS (Electrothermal atomic absorption spectrometry) or FAAS (Flame atomic absorption spectrometry) or ICP-AES (Inductively coupled plasma-atomic emission spectrometry). Metal concentrations were expressed as μg.g⁻¹ dry weight tissue.

Biochemical analysis

MT concentrations in both tissues of mussels and fishes were determined in the heat-treated cytosol by differential pulse polarography in accordance with the method of Thompson & Cosson (1984) and Bebianno & Langston (1989). MT levels were expressed as mg⁻¹ wet weight.

Antioxidant enzyme activities were only determined in the gills and mantle of B. azoricus since the tissues of H. pallidus were collected post mortem and consequently the activity of these enzymes had ceased. Superoxide dismutase (SOD – EC 1.15.1.1), catalase (CAT – EC 1.11.1.6) and glutathione peroxide-dases (GPx) (Fridovich, 1978) including total-GPx and selenium dependent-GPx (Se-GPx – EC 1.11.1.9) were determined by spectrophotometric assays at different wavelengths described by McCord & Fridovich (1969), Greenwald (1985) and Lawrence & Burk (1976) respectively. SOD activity was expressed as U mg⁻¹ of total protein concentrations (one unit of SOD is the amount of the enzyme that inhibits by 50% the reduction of cytochrome c by the xanthine oxidase/hypoxanthine system at a wavelength of 550 nm), CAT as μmoles min⁻¹ mg⁻¹ of total protein concentrations and GPx as nmoles min⁻¹ mg⁻¹ of total protein concentrations.

Lipid peroxidation was also determined only in the gills and mantle of B. azoricus by the method described in Erdelmeier et al. (1998) designed to evaluate malondialdehyde (MDA) and 4-hydroxynonenals (4-HNE). Lipid peroxidation was expressed as μmoles of MDA and 4-HNE g⁻¹ of total protein concentrations.

Total protein concentrations were determined in the gills and mantle of B. azoricus and gills and muscle of H. pallidus by the Lowry method (Lowry et al., 1951) using BSA (bovin serum albumin) as reference standard. Protein concentrations are expressed as mg g⁻¹ wet weight tissue.

RESULTS AND DISCUSSION

The hydrothermal vent mussel B. azoricus shows a great capacity to accumulate metals within their tissues (Table 1). The levels of all metals are considerably higher than those found in coastal mussels like M. edulis or M. galloprovincialis, what strongly reflects the long-term exposure to high metal concentrations from hydrothermal vent fluids. The metal levels
in *B. azoricus* are significantly higher in the gills compared to the mantle, especially for Ag, Cd, Cu and Zn concentrations (p < 0.05). This can be associated to differences in metal uptake routes and specific physiologic functions. In bivalves the metal uptake is mostly done via the gills, as dissolved ions, and via the digestive tract as particles phagocytosed by the digestive gland. Additionally, the mantle acts as reserve storage and secretion of the shell (Fiala-Medioni *et al.*, 2000).

The metal levels observed in *H. pallidus* (Table 1) were significantly lower compared to those found in *B. azoricus* (p < 0.05). Although these fishes depend on hydrothermal vent fauna for food supply, and therefore are frequently found near hydrothermal vents, they have been also captured in non-vent areas (Marques & Porteiro, 2000). This should reduce substantially the exposure time to metals from the vent fluids, comparatively to vent mussels that depend on hydrothermal emissions to maintain the intracellular symbiotic bacteria (both sulphur and methanotrophic) in their gills, which autotrophically synthesize organic matter directly in this tissue and therefore these mussels are continually exposed to the high metal concentration fluids (Southward *et al.*, 2001).

As observed for *B. azoricus*, also in *H. pallidus* the concentrations of most metals (Ag, Cd, Cu and Mn) were significantly higher in the gills, except for Fe (no significant differences between tissues were observed) and Zn (this metal was significantly higher in the muscle) (p < 0.05). This would suggest that chimaera fishes accumulate metals mainly during respiration and these metals are accumulated in the muscle in less extent. Moreover, no evidences of metal bioamplification from mussels to fishes were found.

The MT concentrations in vent mussels (Table 1) were 2-fold higher in the gills (4.6 ± 1.0 mg g\(^{-1}\) w.w.) compared to the mantle (2.1 ± 0.6 mg g\(^{-1}\) w.w.) (p < 0.05). However, higher levels of these proteins would be expected, taking into account the metal accumulation data. Furthermore, MT concentrations in the tissues of *B. azoricus* are close to those reported to their coastal counterparts, exposed to lower metal levels in their environment. This suggests that MT in these molluscs might not be the main detoxification mechanism (Rousse *et al.*, 1998).

Contrarily, in *H. pallidus* MT levels were significantly higher in the muscle (0.27 ± 0.08 mg g\(^{-1}\) w.w.) where lower metal concentrations were found, compared to the gills (0.15 ± 0.02 mg g\(^{-1}\) w.w.) (p < 0.05). These findings were surprising since these proteins are generally associated with the metal detoxification and in this case did not followed the metal accumulation patterns observed in the two tissues, suggesting that, as hypothesized for *B. azoricus*, MT may not be the most important metal detoxification mechanism in chimaera fishes.

Stress related biomarkers were also evaluated in the tissues of *B. azoricus* (Table 2). Generally, the antioxidant enzymatic activities found in this vent mussel are close to those reported for the coastal mussel *M. galloprovincialis* from Ria Formosa lagoon (data not shown). This is surprising as higher levels of antioxidant enzymes would be expected in *B. azoricus* exposed to a great variety of toxic compounds known to enhance reactive oxygen species production from hydrothermal fluids. SOD and CAT activities were significantly higher in the gills, while the activity of both total and Se-GPx was higher in mantle tissue (p < 0.05).

The gills may be more susceptible to oxidative stress, as lipid peroxidation was 2-fold higher in this tissue (187 ± 13 nmol g\(^{-1}\) total proteins) compared to the mantle (99 ± 10 nmol g\(^{-1}\) total proteins).

**CONCLUSIONS**

*B. azoricus* accumulate higher metal concentrations than coastal mussels and chimaera fishes suggesting a long-term exposure to metal rich hydrothermal fluids. The gills are the main target tissue for metal accumulation.

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**Table 1** — Metal (*μg g\(^{-1}\) d.w.) and MT concentrations (mg g\(^{-1}\) prot) in *B. azoricus* and *H. pallidus* from Lucky Strike hydrothermal field. Data are expressed as means ± standard error.

<table>
<thead>
<tr>
<th>Species</th>
<th>Tissues</th>
<th>Ag</th>
<th>Cd</th>
<th>Cu</th>
<th>Fe</th>
<th>Mn</th>
<th>Zn</th>
<th>MT</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>B. azoricus</em></td>
<td>Gills</td>
<td>5.2 ± 1.2</td>
<td>47.2 ± 10.0</td>
<td>79.7 ± 19.4</td>
<td>303 ± 63</td>
<td>7.6 ± 1.9</td>
<td>1976 ± 570</td>
<td>4.6 ± 1.0</td>
</tr>
<tr>
<td></td>
<td>Mantle</td>
<td>0.8 ± 0.2</td>
<td>2.9 ± 1.0</td>
<td>14.2 ± 3.5</td>
<td>235 ± 61</td>
<td>5.8 ± 1.5</td>
<td>124 ± 37</td>
<td>2.1 ± 0.6</td>
</tr>
<tr>
<td><em>H. pallidus</em></td>
<td>Gills</td>
<td>0.6 ± 0.02</td>
<td>0.37 ± 0.1</td>
<td>4.7 ± 0.9</td>
<td>185 ± 43</td>
<td>5.7 ± 1.3</td>
<td>172 ± 48</td>
<td>0.15 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>Muscle</td>
<td>0.08 ± 0.03</td>
<td>0.05 ± 0.01</td>
<td>1.1 ± 0.3</td>
<td>168 ± 48</td>
<td>2.8 ± 1.0</td>
<td>252 ± 101</td>
<td>0.27 ± 0.08</td>
</tr>
</tbody>
</table>

**Table 2** — Activities of antioxidant enzymes and lipid peroxidation levels in the hydrothermal mussel *B. azoricus*.

<table>
<thead>
<tr>
<th>Tissues</th>
<th>SOD (U mg(^{-1}) prot)</th>
<th>CAT (μmol mg(^{-1}) prot)</th>
<th>Total GPx (mmol mg(^{-1}) prot)</th>
<th>Se-GPx (mmol mg(^{-1}) prot)</th>
<th>LPO (nmol g(^{-1}) prot)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gills</td>
<td>19.1 ± 2.9</td>
<td>17.5 ± 4.9</td>
<td>8.7 ± 1.8</td>
<td>6.8 ± 1.1</td>
<td>187 ± 13</td>
</tr>
<tr>
<td>Mantle</td>
<td>14.4 ± 1.6</td>
<td>6.0 ± 1.3</td>
<td>18.5 ± 3.2</td>
<td>15.6 ± 2.1</td>
<td>99 ± 10</td>
</tr>
</tbody>
</table>
Despite the metal concentration antioxidant enzymatic defences are close to those reported in coastal mussels, suggesting a controlled production of ROS in such toxic environment.

MT levels in these mussels are also low, suggesting these proteins are not the major metal detoxification mechanism.

H. pallidus have significantly lower metal levels than the mussels reflecting smaller exposure to hydrothermal fluids and showing no evidences of metal bioamplification.

Like mussels, chimaera fishes accumulate considerably more metals in the gills.

MT, on the other hand, are significantly higher in the muscle tissue not reflecting the metal contamination.

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