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Biological factors influencing tissue compartmentalization of trace metals in the deep-sea hydrothermal vent bivalve *Bathymodiolus azoricus* at geochemically distinct vent sites of the Mid-Atlantic Ridge

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Abstract

In this study, we investigated on concentrations of trace metals (Al, Cd, Mn, Co, and Hg) in the hydrothermal bivalve *Bathymodiolus azoricus,* a dominant species at most vent sites along the Mid-Atlantic Ridge (MAR), and in its endosymbiont bacteria and commensal parasite *Branchipolynoe seepensis*. Comparison of our results with data from the literature on nonhydrothermal bivalves suggests lack of "extreme" uptake of trace metals by *B. azoricus*, except for Hg concentration which exceeded manyfold previously reported values. Mussels collected from three geochemically distinct vent sites, Menez Gwen, Lucky Strike, and Rainbow, along the MAR showed significant differences in tissue concentration of metals. Proportionality of metals in soft tissues of mussels reflected variation of water chemistry at different vents, which in turn conserved the order of trace metal prevalence in undiluted fluids. There were significant tissue-specific differences in trace metal compartmentalization for all metals investigated. Byssus thread contained the highest metal concentration among examined tissues, and thus it is suggested to be an important detoxification route. Size-dependent differences in metal concentrations were detected only for Hg, revealing a general trend of small mussels accumulating more metal than big mussels. Endosymbiont bacteria are shown to exclusively sequester Al from the host gill and contribute to removal of other toxic metals in mussels from Menez Gwen. The commensal parasite present in all mussels from Lucky Strike had higher tissue concentrations of Mn, Al, and Co than the host gill, unlike Cd and Hg which were considerably lower in the former, and thus its role in detoxification remains unclear. Bioaccumulation potential of vent bivalves and associated organisms are quantified as concentration factors and compared to make inferences on the putative role of the endosymbiont bacteria and the commensal parasite in detoxification of trace metals.

Keywords: Hydrothermal vent; Mixing zone; Heavy metals; *Bathymodiolus azoricus*; *Branchipolynoe seepensis*; Endosymbiont bacteria

1. Introduction

Offshore hydrothermal vents occur worldwide at sea-floor-spreading centers (Fornari and Embley, 1995) as a result of the convective circulation of seawater through newly formed oceanic rifts. Mineral-rich water emitted from these areas with elevated methane, hydrogen, and geothermally reduced sulfide concentrations mixes with the ocean water, forming a dynamic system with physico-chemical factors shifting over very short spatial and temporal scales (Sarradin et al., 1999). These interfacial zones host typical hydrothermal communities that are likely to provide unique natural laboratories to study special regulation mechanisms supporting survival under these extreme conditions. Across a diverse spectrum of organisms, the absorption and toxicity of trace elements are powerful laboratory tools to assess the impact of industrial man on world ecosystems. For the first time, it is possible to study concentrations of various trace elements (Al, Cd, Co, Hg, and Mn) in aquatic biota that are naturally coexposed to a variety of these metals. They have all been identified as toxic for living organisms, even when present at low levels (Wexler, 1998). There is an

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ever-growing literature on detailed description of their toxicokinetics, their toxic effects on living organisms, and their admissible exposure limits, whose description is beyond the scope of this study.

Many macroorganisms from hydrothermal vents have developed symbiotic relationships with chemoautotrophic bacteria to utilize the chemical energy of this deep-sea environment (Cavanaugh, 1983; Fiala-Medioni et al., 2002). The hydrothermal mussel *Bathymodiolus azoricus* (Von Cosel et al., 1999) which is dominant in many of the vent sites of the Mid-Atlantic Ridge (MAR) is functionally dependent on the association with both sulfur oxidizer and methanotroph bacteria (Robinson et al., 1998; Kádár et al., 2005a). It has been shown to accumulate high levels of heavy metals, the main target organs being the gill and digestive gland (Rousse et al., 1998; Fiala-Medioni et al., 2000). Differences in tissue metal concentrations of *B. azoricus* from different vent sites were suggested to be attributed to distinct fluid chemistry at their habitat (Rousse et al., 1998).

The three vent sites along the MAR that were chosen as sampling sites for this study owing to their distinct geochemical setting are: Menez Gwen (−850 m, 37°50′N, 31°31′W) (Charlou et al., 2000), Lucky Strike (−1700 m, 37°18′N, 32°16′W) (Langmuir et al., 1997), and Rainbow (−2300 m, 36°13′N, 33°54′W) (Douville et al., 2002). Detailed description of these vents and clear geochemical differences in their fluid composition were reported in the literature as a result of over a decade of extensive investigation (Charlou et al., 2000; Desbruyeres et al., 2001; Sarradin et al., 1999). However, data on metal bioavailability in the mixing zone where mussel clumps are thriving are lacking to date, owing to sampling difficulties associated with such dynamic systems. Curious to know whether water metal concentrations are reflected in the local fauna, tissue concentration of five trace metals (Al, Cd, Co, Mn, and Hg) in various tissues of *B. azoricus* and its surrounding environment were investigated. Organisms–metal interactions, with special view on biological factors (host–symbiont and host–parasite interactions) that may influence bioavailability of metals were also envisaged. Bioaccumulation of toxic metals in endosymbiont bacteria (both sulfur- and methane-oxidizers) and in the commensal parasite (*Branchipolynoe seepensis*) was investigated and concentration factors for individual metals indicative of the bioaccumulation potential were calculated and discussed in correlation with available data from the literature on bivalves inhabiting contaminated sites.

2. Materials and methods

2.1. Sample collection

Samples were collected during the SEAHMA I cruise between 29 July and 14 August, 2002, with the R/V Atalante, using the equipment secured to the Remotely Operated Vehicle (ROV) Victor 6000.

Discrete water samples were taken above sampled mussel and shrimp microhabitats in each vent site (M. Gwen, L. Strike, Rainbow) using the multisampler unit (PEP) of Victor that houses 19 200-mL titanium syringes associated with an autonomous temperature probe. Samples were acidified with high-purity HNO_3 to pH 2 and kept refrigerated in high-density polyethylene vials until analysis.

Mussels of different size ranges (group 1 with shell length between 2 and 5 cm, group 2 with 5–7 cm, and group 3 with shell larger than 7 cm) were collected from M. Gwen, L. Strike, and Rainbow vent sites by the telemanipulated ROV arm and brought to the surface in an insulated box. Individuals were measured and dissected freshly into the main organs of heavy metal storage, gill, digestive gland, mantle, and byssus, and kept frozen until dehydration, acid digestion and analysis.

Parasitic scale worms from mussels from L. Strike were individually removed from gills and whole specimens were frozen until preparation for analysis. Worms were absent in mussels sampled at M. Gwen and Rainbow.

All concentrations throughout the text, unless otherwise stated (whether mussel tissue, purified bacteria, or polychaete tissue), are given per unit of dry weight.

2.2. Bacterial isolation

Endosymbiont bacteria were purified from mussels 24 h after being brought to the surface, using the technique described by Distel and Felbeck (1998). Briefly, a gill homogenate was obtained by crushing the freshly removed gills with the addition of 3 mL imidazol-buffered saline (IBS) buffer/g of wet weight tissue on a laboratory tissue grinder (Ystral D-79282; Ballrechten-Dottingen). The homogenate was centrifuged with an equal volume of the mix of Percoll with 2.5 × diluted IBS, (v/v 6:4) using an Eppendorf swinging bucket rotor at 10,300 rpm for 6 min at 4 °C. Bacterial pellets were recovered using Pasteur pipettes and then lyophilized in a Savant refrigerated vapor trap system overnight. Dried samples were pooled for 10 mussels and kept in a desiccator until acid digestion and further analysis. Cells in bacterial preparations were routinely inspected for homogeneity and/or potential contamination using a Zeiss phase-contrast microscope. Because of the timing of the scientific mission which permitted transportation only of fresh mussels to the laboratory from the M. Gwen vent site, bacteria were not extracted from mussels from L. Strike.

2.3. Multielement analysis in water samples and tissue digests

Seawater samples were prepared and analyzed using an Elan DRC ICP-MS system (Perkin–Elmer, Beaconsfield, UK) and an analytical protocol described elsewhere (Kádár et al., 2005). For quality control the following reference materials (artificial seawater) were analyzed: CRM 403, CRM 403 spiked with 5 $\mu g\,L^{-1}$ of most elements, diluent, and diluent spiked with 5 $\mu g\,L^{-1}$ of all elements. Recoveries of spiked reference material were as follows: 117.8% for Al, 106.13% for Mn, 92.6% for Cd, and 82.9% for Co.

Tissues and bacterial cell suspensions were acid digested prior to analysis as previously described (Jugdaohsingh et al., 1998). Briefly, 0.5–1 g of dry tissue (previously lyophilized using a Savant refrigerated vapor trap system overnight) was digested with equal volumes (1 mL/0.1 g dry weight) of Aristar-grade concentrated (69% v/v) nitric acid and 30% hydrogen peroxide for 24 h. Digested samples were diluted 1:4 with high-purity deionized water prior to analysis on the above-described ICP-MS instrument. Standards were prepared using a commercially available multielement standard added to blank digests and ranged between 0 and 2000 $\mu g\,mL^{-1}$ for all metals of interest. The 0 $\mu g\,mL^{-1}$ solution was used as a blank. Accuracy of analytical method was monitored by analyzing two different certified reference materials, CRM 414 (plankton) and CRM 278R (mussel tissue), and, in both, measured values were within 12% for Zn, Cu, and Cd. Concentration of Al in these reference tissues is not available and Co is given only as not certified in CRM 414.