



# Metal compartmentalization and metallothionein isoforms in mussels from the Mid-Atlantic Ridge; preliminary approach to the fluid-organism relationship

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## Introduction

Hydrothermal fluids are characterized by low pH, high hydrogen sulphide concentrations, anoxia and a high metallic content. Vent metazoans live in a mixing zone whose characteristics are less harsh than the fluids themselves, but they are still more extreme than those conditions typical of the rest of the deep sea water. To survive in such an environment, they have developed specific physiological adaptations which are still under investigation.

The present work was designed as a first approach to the relationship between heavy elements present in the water surrounding the organisms and their potential biological impact. Our research on the essential and toxic metals focuses on their concentration and bioavailability in the ambient medium, their bioaccumulation by the organisms and the detoxification mechanisms developed by these organisms. Our study of heavy metals (Cd, Cu, Zn) and metallothionein isoforms in the mussel *Bathymodiolus* sp. from the Mid-Atlantic Ridge is part of a global study on trace element bioaccumulation by hydrothermal vent mytilids.

Metallothioneins are low molecular weight proteins involved in metal (Zn, Cu) intracellular regulation whose participation in metal detoxification is well established. Organ-specific isoforms have been reported in most of the studied species but little is known about their respective functions (for review see Suzuki et al., 1993).

## Materials and methods

Twelve mussels were collected during the Diva2 cruise (1994) at the 'Eiffel Tower' and 'Elizabeth' sites in the Lucky Strike area and kept frozen (-80°C) until analysis.

Water samples were collected around the organisms, using 750 ml titanium syringes to establish a relationship between individual metallic impregnation and the corresponding background chemical parameters.

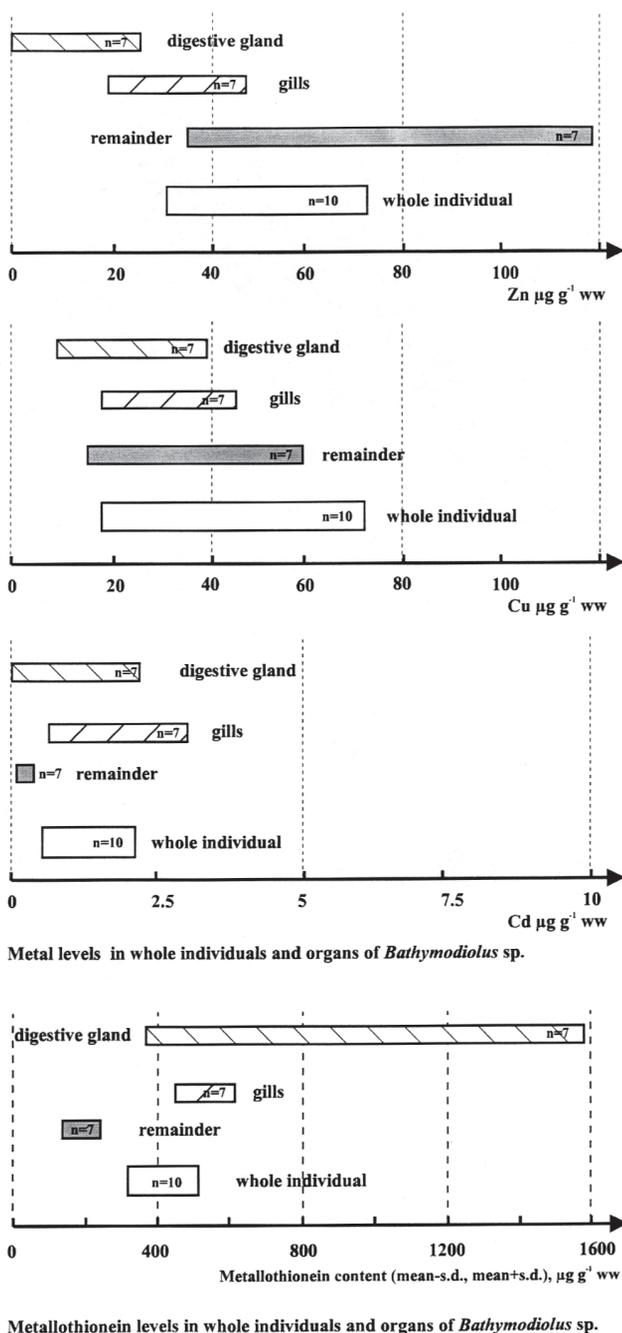
The digestive gland, a major organ for metabolism, and the gills which are in direct contact with the ambient medium and harbour symbiotic bacteria, were separated from the remaining tissues (known to play a minor role in metal detoxification processes), except for three small mussels from 'Elizabeth' site, which were analysed whole.

To determine the heavy metal balance within subcellular fractions and the metal-binding protein levels, samples were partially thawed and homogenized in a Tris buffer on ice before centrifugation. Pellets and aliquots of supernatants were digested with nitric acid for metal analysis. Remaining supernatants were subjected to heat-denaturation and centrifuged. Pellets and supernatants obtained after this second centrifugation were digested (as above), or frozen, until metal or metallothionein quantification, respectively.

Copper and lead in acidified water samples were determined by anodic stripping voltametry (Sarradin et al., submitted; Riso et al., 1997). Metal and metallothionein analyses in biological tissues were performed by atomic absorption spectrophotometry and differential pulse polarography respectively (Cosson & Vivier, 1997). Metallothionein isoforms were separated using anion exchange-HPLC with a diethylaminoethyl (DEAE) column, or reversed phase-HPLC with a C18 column.

## Results and Discussion

The water surrounding the mussels is highly enriched in Cu and Pb (total dissolved metal) with concentrations ranging from 1.3-130 µg l<sup>-1</sup> Cu and 200-3300 ng l<sup>-1</sup> Pb at 'Eiffel Tower' site and from 14-22.2 µg l<sup>-1</sup> Cu and 1240-2280 ng l<sup>-1</sup>



**Figure 1.** Metals and metallothionein levels in whole individuals and organs of *Bathymodiolus* sp.

Pb at 'Elizabeth' site. These data appear in Table 1. This large concentration variability can be linked to the strong affinity of those metals to form sulphide precipitates. Godfrey et al. (1994) observed that lead concentrations decreased rapidly away from the source because of its precipitation as lead sulphide. Both metals behave like Mn, Fe, Co, Zn, Cd and Hg (Von Damm, 1983) and will form

insoluble precipitates with sulphides in plumes (Trefry & Trocine, 1985), and on chimneys and conduit surfaces. A comparison of these values with the concentrations of copper reported for polluted and unpolluted water bodies shows that hydrothermal organisms are subjected to Cu and Pb concentrations up to 1000 times greater than in unperturbed environments.

**Table 1.** Concentrations ranges of Cu and Pb in water samples

|                      | Cu, ppb ( $\mu\text{g l}^{-1}$ ) | Pb, ppt ( $\text{ng l}^{-1}$ ) |
|----------------------|----------------------------------|--------------------------------|
| bottom seawater      | 0.1-0.3                          | 1-4                            |
| surrounding mussels  | 1.3-130                          | 200-3300                       |
| estimated pure fluid | 800-1000                         | 30,000-40,000                  |

Mean levels (standard deviation) calculated or directly measured in the whole organisms were 1.12 (0.82), 45.7 (30.5), 47.5 (20.6) mg of metal per kg of wet weight ( $\text{mg kg}^{-1}$  w.w.) for cadmium, copper and zinc respectively. Variation coefficients were very high showing the dispersion of the data. This variability could be explained by differences in the physiological status of the mussels and by their location relative to the vent. Cadmium was preferentially located in the gills and its level in the remainder was low. Copper was found in equal proportions in the gills and the remainder, its level being higher than in the digestive gland. Zinc was mostly present in the gills and equally encountered in the digestive gland and in the remainder. Metals were preferably bound to insoluble compounds with percentages varying with the considered anatomic part. Copper showed a higher affinity for soluble compounds than cadmium and zinc.

The mean level of metallothioneins was  $417 \text{ mg kg}^{-1}$  w.w. for the whole body. However, the mean metallothionein level in the digestive gland ( $969 \text{ mg kg}^{-1}$  w.w.) was twice that of the gills ( $530 \text{ mg kg}^{-1}$ ). The mean level in the remainder was low ( $186 \text{ mg kg}^{-1}$ ).

Isoforms of metallothioneins were resolved by ion exchange-HPLC in two groups (MT1 and MT2) in the three tissues, as generally observed for bivalves (Mackay et al., 1993). Isoforms of the MT1 group were predominant in the digestive gland samples.

Reversed-phase-HPLC separation of these isoforms and the spectral identification of the associated metal were not accurate enough to characterize the separated peaks.

Concentrations factors (CF) were calculated to estimate the bioaccumulation by the organisms (Table 2). These CF are in the same range as those found for the oyster *Crassostrea gigas* Thunberg, 1793, exposed for 12 days to  $50 \mu\text{g Cu l}^{-1}$  leading to a CF of 49,200. (Amiard-Triquet et al., 1988).

**Table 2.** Concentrations factors for copper.CF=[Cu<sub>organisms</sub>]/[Cu<sub>water</sub>].

|    | whole organism | gills      | digestive gland | remainder  |
|----|----------------|------------|-----------------|------------|
| CF | 350-35,000     | 250-25,000 | 190-19,000      | 290-29,000 |

### Conclusion

Variation in environmental levels of heavy metals, coupled with possible intraspecific metal-uptake differences, leads to significant variation in heavy metal bioaccumulation by *Bathymodiolus* sp. The high concentration factor of copper and the high levels of metallothioneins in the analysed tissues are consistent with the hypothesis of heavy metal bioaccumulation by *Bathymodiolus* sp. The storage of metals in the insoluble form suggests that this detoxification pathway has the same importance in *Bathymodiolus* sp. as in other (non-vent dwelling) bivalves.

The following step of this study must employ a multidisciplinary approach involving chemistry, ecology, ecotoxicology, and histology on a limited and well defined study area using a combined sampling strategy (organisms, water, temperature) to obtain a better understanding of the physical and chemical parameters of the water surrounding the organisms and, in addition, to determine a greater number of characteristic metals (Ag, Cd, Cu, Hg, Pb, Zn). Investigations are underway using liquid chromatography-electrospray ionisation-mass spectrometry (LC-ESI-MS) and liquid chromatography-inductive coupled plasma-mass spectrometry (LC-ICP-MS) to determine the amino acid sequences and the nature of the associated metal of each isoform.

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