Marine Micropaleontology 126 (2016) 42–49

Contents lists available at ScienceDirect

Marine Micropaleontology

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

Research paper

Zinc incorporation in the miliolid foraminifer *Pseudotriloculina rotunda* under laboratory conditions

M.P. Nardelli [a,b,*], D. Malferrari [c], A. Ferretti [c], A. Bartolini [d], A. Sabbatini [a], A. Negri [a]

[a] Department of Life and Environmental Sciences (DiSVA), Politechnic University of Marche, Ancona, Italy
[b] UMR CNRS 6112 LPG-BIAF, University of Angers, Angers, France
[c] Department of Chemical and Geological Sciences, University of Modena and Reggio Emilia, Modena, Italy
[d] Centre de Recherche sur la Paléobiodiversité et les Paléoenvironnements, UMR 7207 CNRS MNHN UPMC, Muséum National d'Histoire Naturelle, Paris Cedex 05, France

ARTICLE INFO

Article history:
Received 7 July 2015
Received in revised form 30 May 2016
Accepted 5 June 2016
Available online 6 June 2016

Keywords:
Foraminifera
Miliolid
Zn/Ca
LA-ICP-MS
Culture experiments
Pollution

ABSTRACT

The incorporation rate of Zn into the calcareous tests of *Pseudotriloculina rotunda* was investigated in culture in order to evaluate the possibility of using Zn/Ca ratios as a pollution proxy. Foraminifera were incubated at zinc concentrations up to 10-fold higher than unpolluted seawater (sea + 10 mg Zn/L) during 70 days. New calcite was investigated under the Environmental Scanning Electron Microscope (ESEM), for potential alteration of test structure. Laser ablation-Inductively Coupled Plasma-Mass spectrometry (LA-ICP-MS) was used to quantify Zn contents. The analyses revealed that test structure is not visibly altered by the presence of zinc. However, significant Zn incorporation is detected by the LA-ICP-MS. The zinc partition coefficient, D_{Zn}, decreases at increasing Zn concentrations (from 4.03 ± 0.06 to 0.2 ± 0.01) and the zinc is incorporated into the calcite not necessarily linearly.

1. Introduction

The recent worldwide legislation aims to restore the "pre-anthropic impacts" status in marine environments (e.g., WFD, 2000/60/EC and MSFD, 2008/56/EC in Europe). Information about the pristine faunas like those in pre-industrial times, however, are often impossible to obtain because of the scarcity (or lack) of reference stations that could still represent unimpacted present-day conditions. Fossilizing organisms represent an excellent historical archive of environmental conditions. Foraminifera are distributed worldwide in many different habitats, from brackish to marine, and their fossil records create an excellent historical archive which can be used as proxies for the reconstruction of past environments, such as pre-industrial ecological conditions (Schönfeld et al., 2012). A new approach involves the use of foraminiferal test geochemistry to assess the evolution of pollutant (i.e., metals) concentrations through time. Incorporation rates of trace elements are widely used as specific proxies in paleoceanography and paleoecology (e.g., Eggins et al., 2003; Hönisch and Hemming, 2005; Levi et al., 2007; Katz et al., 2010; Sabbatini et al., 2011), despite the possible bias linked to the biological influence on calcification processes (i.e., vital effects). The need to calibrate these proxies through culturing experiments was highlighted in the last decade by several authors (e.g., de Nooijer et al., 2007). This approach offers the advantage of changing one single variable (while all the others are kept constant) in order to better evaluate the vital effect. These biological aspects could be even more important for the incorporation rates of chemicals whose concentrations exceed natural baselines due to human activity, and that could be potentially used as pollution markers.

In this study we investigate the incorporation rates of Zn in the shell of the benthic miliolid foraminifer *Pseudotriloculina rotunda* (Schlumberger, 1893). Among foraminiferal species miliolids showed contradictory responses to heavy metal pollution in different studies. For example, decreasing miliolid relative abundances in polluted (by both organic and inorganic chemicals) coastal zones are reported and interpreted by some authors as a sensitivity index (e.g., Ferraro et al., 2006; Frontalini and Coccioni, 2008). Other studies, on the other hand, suggest a strong tolerance of several miliolid species to pollution, both in situ and under laboratory conditions (e.g., Samir and El-Din, 2001; Romano et al., 2008; Cherchi et al., 2009; Foster et al., 2012; Nardelli et al., 2013).

The aim of the present study is to calibrate incorporation rates of Zn in miliolid foraminiferal shells and thus evaluate the usefulness of the Zn incorporation rate as an environmental proxy. Zn is, in fact, one of the most common pollutants associated with human activities (e.g., Callender and Rice, 2000; Wuana and Okieimen, 2011), that can be toxic for biological systems when its concentration exceeds a threshold value (e.g., Haase et al., 2001; Valko et al., 2005; Díaz et al., 2006; Formigari et al., 2007). Nardelli et al. (2013) showed that inorganic Zn

* Corresponding author at: UMR CNRS 6112 LPG-BIAF, University of Angers, 2 Boulevard Lavoisier, 49045 Angers Cedex, France.
E-mail address: mariapia.nardelli@univ-angers.fr (M.P. Nardelli).

http://dx.doi.org/10.1016/j.marmicro.2016.06.001
0377-8398/

at concentrations higher than 0.1 mg/L can cause biological stress in *Pseudotriloculina rotunda*, causing delay in calcification rates.

In this regard, our study also aims to test the hypothesis that the biological stress caused by high Zn concentrations may influence metal incorporation rates as well. Zn incorporation was investigated using Laser Ablation Inductively Coupled Plasma Mass Spectrometer (LA-ICP-MS) analysis. Moreover, morphological observation using the Environmental Scanning Electron Microscope (ESEM) was employed to check for abnormalities in the organization/distribution of single shell crystallites. Nardelli et al. (2013) previously suggested that Zn does not cause macroscopic test deformations in miliolid foraminiferal shells, on the base of their observation of coiling patterns and chamber shapes of *P. rotunda* specimens grown at increasing zinc concentrations, under the binocular microscope. The aim of our ESEM analyses was to deepen these observations and check for calcite anomalies at the crystallite level.

2. Material and methods

2.1. Experimental set up

All the analyses were performed on specimens of *Pseudotriloculina rotunda* that grew at least one chamber during 70-days exposure to six different Zn concentrations under laboratory conditions. Zn-enriched solutions were prepared adding respectively 0.01, 0.1, 1.0, 10, and 100 mg/L of Zn to natural seawater (sea) from an unpolluted site (Portonovo, Adriatic Sea); Zn and Ca concentrations in natural seawater were measured using an Inductively Coupled Plasma Mass Spectrometer (ICP-MS). Although precautions were taken during manipulations, the ICP-MS analyses revealed that the zinc concentration of the natural seawater used for the experiment was higher than natural background. In fact the zinc concentration measured on culture waters before the addition of zinc was 0.149 ± 0.01 mg/L, while the zinc concentration of seawater from the sampling site was originally 0.237 ± 0.01 μg/L. However, as the same water was used to prepare all the zinc solutions for the different treatments and, considering the very high concentrations of added zinc, we believe that this contamination does not compromise the dataset. But, of course, this means that the lowest tested seawater zinc concentration of the experiment (named "sea" hereafter) cannot be considered as a control representative of unpolluted seawater conditions.

Temperature (15.0 ± 0.5 °C), salinity (38.0 ± 0.001) and pH (8.0 ± 0.1) were kept constant during the experiment. Refer to Nardelli et al. (2013) for further details on culture settings and preparation of Zn solutions.

As reported in Nardelli et al. (2013), none of the specimens produced new chambers at the highest tested Zn concentration (sea + 100 mg/L), therefore only specimens coming from culture sets from treatments sea, sea + 0.01, sea + 0.1, sea + 1 and sea + 10 mg/L were investigated. Moreover, two samples were treated for a "passive Zn incorporation test": empty tests of *P. rotunda* were exposed to sea + 10 mg/L Zn concentrations for two weeks in order to measure Zn passively adsorbed to calcite, without involving cellular-mediated mineralization processes.

2.2. ESEM and LA-ICP-MS sample preparation

The analyzed foraminiferal tests (n = 41) were washed with Millipore water and dried at 40 °C. The same cleaning procedure was performed on the two empty tests (n = 2) used for the passive incorporation test. To perform both ESEM observations and LA-ICP-MS analyses, samples were fixed to aluminium stubs using conductive carbon adhesive discs. All samples analyzed with an LA-ICP-MS were photographed under the ESEM before and after the analyses to check for the success of ablations (i.e., the correct chamber, no multiple chamber sampling, and no breakage of chambers – see Appendix, Fig. A.1a–c).

2.3. LA-ICP MS analysis: analytical protocol optimization

This study represents the first LA-ICP-MS investigation on miliolid foraminifera. Chemical composition, micro-structure and chamber arrangements of miliolids strongly differ from other benthic foraminifera more commonly analyzed with the LA-ICP-MS (i.e., Rotaliidae, e.g., de Nooijer et al., 2007; Munsel et al., 2010; Dissard et al., 2010; or Buliminidae, e.g., Hintz et al., 2006; Barras et al., 2010). Miliolid foraminifera have a calcareous non-lamellar imperforate test consisting of calcite needles randomly oriented in an organic matrix. They also possess a smoothly finished outermost layer of well crystallized calcite, with rhombohedral crystal faces arranged parallel to the surface (Debenay et al., 1998). Moreover, *Pseudotriloculina rotunda* creates chambers, each one-half coil in length, adding the new ones in planes oriented at 120°, with only three final externally visible chambers (Loeblich and Tappan, 1964).

For this reason it was necessary to optimize the existing protocols and to obtain the best ablation setting to be applied to our specimens. In particular, it was compulsory to prevent the laser from ablating the innermost (older) chambers. Several trials on foraminiferal tests were thus performed to find the most suitable combination of the instrument setting parameters. A detailed description of the followed procedures is given in Appendix A, and the values of the optimized parameter used for measurements are reported in Table 1. An example of successful sampling is shown in Fig. 1.

2.3.1. Analytical standards preparation and instrument calibration

A mass spectrometer, like any measurement device, requires a suitable calibration procedure. When laser ablation is employed, the interaction between laser and solid sample is complex and the response is dependent on the sample matrix. For this reason, two forms of calibration are mandatory: i) a reference (internal standard) is required to compensate for changes in the quantity of ablated mass, even when the concentration remains constant; ii) matrix-matched solid standards (frequently referred to as "external standards") are necessary to calibrate laser ablation processes and the instrument response. In fact, a relative measure of ablated mass can be achieved by simultaneously measuring emission from the analyte and a common matrix element (internal standard). For absolute calibration of the LA-ICP-MS conditions, standards made of the same matrix as the samples would be required, but are seldom available (e.g., Darke and Tyson, 1994; Raith et al., 1996; Hathorne et al., 2003).

Table 1
Optimized laser parameters. Linear ablation, carried out on the flatter part of the last chamber proceeding always from the center to the aperture of the chamber, was preferred to spot ablation. See Hathorne et al. (2003) for comparison and SI-1 for further information. A pre-ablation step was introduced to clean the surface from potential contaminations before data acquisition.

	Laser intensity (%)	Frequency (Hz)	Ablation line width (μm)	Duration (s)	Main results
Pre-ablation	10	1	55	230	Sampling only of external chamber, without crash
Ablation	50	4	55	230	Good signal intensity at the detector