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Zinc association with surface-bound iron-hydroxides on cultured marine diatoms: A zinc stable isotope perspective

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ABSTRACT

Uptake of trace metals by marine phytoplankton for metabolic use exerts a fundamental control on their marine geochemical distributions. Moreover, such trace metals limit primary productivity over large areas of the surface ocean. As such, an understanding of the mechanisms and extent of phytoplankton uptake are essential components of oceanic trace metal chemistry. Efforts to quantify intra-cellular quotas of phytoplankton are complicated by the presence of metals adsorbed to external surfaces, including surface-bound Fe-hydroxides, both in nature and in culturing experiments. In the relatively new discipline focused on oceanic metal isotopes, these surface-bound metal hydroxides may be of particular importance in that they could result in isotope signatures that complicate studies that seek to understand intra-cellular signatures related to metabolic uptake.

In this contribution, we assess the extent to which heavy Zn isotopes are preferentially adsorbed to surface-bound Fe-hydroxides on marine diatoms. For this purpose, the marine diatom *Thalassiosira oceanica* has been cultured at low *versus* high inorganic Fe concentrations in the medium, while two further diatoms strains have been compared at elevated Fe levels. The formation of surface bound Fe-hydroxides at elevated Fe was further stimulated by reducing the trace metal buffering capacity of the experimental medium, lowering the concentration of the used organic chelator. We also investigate an alternative procedure for quantifying intra-cellular metal quotas, the analysis of the contents of deliberately lysed cells.

In good agreement with previous work, we find that biomass associated Fe/P ratios represent a good proxy for the absolute quantity of Fe-precipitates on diatom surfaces. Zn sorption to these surface-bound Fe-hydroxides can drive bulk biomass δ^{66}Zn compositions up. On the other hand, the loss of heavy Zn from the experimental medium causes the biomass Δ^{66}Zn, if referred to the starting medium, to be biased in the other direction, towards more negative values. To avoid any such complications, likely to occur at high Fe or low buffer capacities, we conclude that diatoms cultured at low Fe are most likely to record the Δ^{66}Zn signatures of Zn uptake into the phytoplankton cell.

Taxonomy

Trace Metal
Isotope Geochemistry
Phytoplankton
Biogeochemistry
Oceanography
Adsorption

1. Introduction

Extreme scarcity of Zn and Fe in the euphotic zone, coupled to deep enrichments (Bruland et al., 2014), is consistent with biological uptake at the surface and regeneration at depth. It is well-established that transition metals such as zinc (Zn) and iron (Fe) are required by marine phytoplankton as essential micronutrients (for a review, see Morel et al., 2014). Both elements fulfill important structural functions, often as cofactors in enzymes required for growth, for the fixation of atmospheric CO_2 (Zn) into biomass (Morel et al., 1994), and for electron transport (Fe) within the cell (Greene et al., 1991). The physiology of diatoms, which accounts for as much as 20% of carbon fixation on Earth (Armbrust, 2009), is particularly closely linked to the bioavailability of these metals (Anderson et al., 1978; Harrison and Morel, 1986; Sunda and Huntsman, 1992; Sunda and Huntsman, 1997). A recent modeling study has suggested that diatom uptake, particularly in the Southern Ocean, dominates the oceanic cycle of Zn (Vance et al., 2017).

Recently, efforts have begun to harness the power of stable isotopes (*e.g.* for Zn, Conway and John, 2014; Zhao et al., 2014) to unravel the

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oceanic biogeochemical cycling of transition metals, including the impact of biological uptake and regeneration. The first studies have revealed a remarkable homogeneity in Zn isotopes beneath the permanent thermocline, with variable signatures confined to the euphotic zone (Conway and John, 2014; Zhao et al., 2014). In addition to the measurement of isotopic signatures in samples of the real ocean, the exploitation of stable isotopes demands an understanding of the isotope fractionations that accompany key oceanic processes, including those of biological origin. Thus far, only two culturing studies have been performed to study isotope fractionation upon uptake of Zn into marine diatoms (John et al., 2007; Köbberich and Vance, 2017). Further culturing evidence, elucidating key controlling mechanisms that might potentially cause the observed variability in the upper ocean, is required. However, culturing approaches, that aim to study the distribution of metal isotopes between marine diatoms and seawater, might be complicated by experimental difficulties, some of which are addressed in this contribution.

Of prime interest is the isotopic signature associated with cellular uptake in the photic zone, a signature that is ultimately transferred to the deep ocean, and regenerated there, by export productivity. In order to isolate and characterize this signal, culturing studies must maintain the integrity of the isotopic signature internal to the cell right up to the point of analysis. Complications can arise from the potentially strong tendency of metals to sorb to external cell surfaces (Gélabert et al., 2006), resulting in the requirement to remove this metal pool (Hudson and Morel, 1989) while preserving the intra-cellular pool for analysis. Transition metals in artificial culturing media are commonly well-buffered by strong organic chelators, such as EDTA (Anderson and Morel, 1982; Sunda et al., 2005). This is done to keep the inorganically bound, bioavailable, metal concentration at a low, and constant, level over the duration of the culturing experiment. However, the fast oxidation kinetics of ferrous iron in ventilated waters can lead to the formation of colloidal or solid Fe-hydroxides phases (Stumm and Sulzberger, 1992) that tend to nucleate on surfaces, including those provided by marine phytoplankton (Gélabert et al., 2006). Zn has a high affinity for the surface sites of solid Fe-oxyhydroxides (Dzombak and Morel, 1990), a process that should be accompanied by the preferential adsorption of heavy Zn isotopes (Gélabert et al., 2006; Juillot et al., 2008). Pioneering work by John et al. (2007), the first to explore the fractionation of Zn isotopes associated with uptake into a marine diatom, dealt with this difficulty by applying a cleaning procedure designed to remove surface bound Fe-hydroxides (Tovar-Sanchez et al., 2003), including Zn adsorbed to them, in order to isolate uptake related isotope effects (John et al., 2007).

The currently most accepted protocol for the removal of cell surface-bound Fe-oxyhydroxides and other elements associated with them (Hudson and Morel, 1989) relies on reductive dissolution. However, the study of stable metal isotopes introduces special challenges in that all cleaning reagents are required to have low metal blanks. Thus, the John et al. (2007) study used a novel cleaning protocol suggested by Tovar-Sanchez et al. (2003), involving oxalate as a reducing agent. The advantage of this approach is that a liquid-liquid extraction can be used to reduce metal blanks to sufficiently low levels (Tovar-Sanchez et al., 2003). Tang and Morel (2006), however, rigorously compared results for such a strong reductive cleaning reagent versus rinsing with a metal clean sodium chloride solution. The results are striking in that, for a range of metals, including those that are currently subject to isotope studies such as Zn, Cd, Cu, the two methods produce almost identical metal to phosphorus (P) ratios in the final cleaned cells at low Fe concentrations, suggesting that the two cleaning procedures isolate the same intra-cellular metal pool. These findings raise the important question of whether reductive cleaning, in culturing studies that aim to study metal isotopes, is necessary at all.

In this contribution, we seek to elucidate the impact of surface-bound Fe-hydroxides on the distribution of Zn isotopes in cultured marine diatoms. Specifically, we have three main objectives. Firstly, we aim to compare Zn isotope fractionation between three distinct diatom strains and their culturing medium, all cultured at elevated Fe and low chelator concentrations to induce large quantities of surface-bound Fe-hydroxides. Secondly, we aim to investigate whether variable total Fe levels in the culturing medium influence Fe-hydroxide formation on diatom surfaces, including the degree to which these might affect the Zn isotope compositions of the bulk biomass. Thirdly, we evaluate a novel method, which deliberately lyses the cells for analysis of the products, with the aim of quantifying intra-cellular isotope ratios in a potentially more robust manner than established procedures. The comparison of diatoms treated with this newly developed approach with cells rinsed only with metal-free seawater aims to address the maximum impact that elevated quantities of surface associated Fe-hydroxides might have on the distribution of Zn isotopes associated with cultured marine diatoms.

2. Materials & methods

All reagents and solutions used in this study were either of trace metal purity or were cleaned using a chelating resin (Chelex® 100, Bio-Rad, USA). Reagent grade acids were twice purified by sub-boiling distillation (DST-1000, Savillex, USA). 18.2 MΩ·cm ultrapure water came from a Milli-Q® integral water purification system (Merck, Millipore, Germany). Preparation of artificial seawater, manipulation of phytoplankton cultures, and handling of all samples was carried out under 'Class 100' clean laboratory conditions at constant humidity of around 10%, a temperature of 21.2 ± 0.2 °C, maintaining sterile techniques. All stock solutions required for culturing were stored exclusively in fluorinated ethylene propylene (FEP) bottles, preventing the sorption of metals to the bottle. Furthermore, this material allows rigorous cleaning which helps to prevent contamination.

2.1. Diatom culturing

Three different axenic diatom strains were obtained from the National Center for Marine Algae and Microbiota (NCMA), formerly known as Provasoli-Guillard Center for Culture of Marine Phytoplankton (CCMP), Bigelow Laboratories, USA. The *Thalassiosira* strains, *T. oceanica* (CCMP 1005), *T. pseudonana* (CCMP 1015), and *T. weissflogii* (CCMP 1336) were cultured at a temperature of 24 °C ± 1 °C. Light was supplied to phytoplankton cultures in 15- to 9-h light to dark cycles with an hour long gradual dusk and dawn. 24 W Master TL5 HO fluorescent light bulbs (Philips, Germany) of known light spectrum (near natural, low UV) were used for all culturing experiments. Photosynthetically active radiation (PAR) was mapped with a spherical quantum sensor LI-193 (LI-COR®, Nebraska, USA), achieving an angular response of 82% as estimated by deviation from the sensor's azimuth. Photon flux densities of 50 $\mu mol\, m^{-2}\, s^{-1}$ have been achieved by varying the distance to the light source. All phytoplankton strains were illuminated and cultured at the media compositions used later for experimentation. This was done for at least 10 transfer cycles prior to the experiment, and aimed at complete physiological acclimation of the cell to the prevailing experimental conditions.

The artificial culturing medium developed for this study aims to vary Fe over a wide range of concentrations at elevated, but comparable, Zn levels. This was done to study the impact of diatom surface-bound Fe precipitates on the distribution of Zn isotopes between the culturing medium and biomass-associated Zn.

The seawater base and vitamin supplements of the artificial medium used here are identical to those described by Berges et al. (2001), a medium sometimes referred to as ESAW. Whereas the major nutrients nitrate and phosphate were also as given in Berges et al. (2001), silicate concentrations were kept at a slightly lower level (Provasoli, 1968). Including all additions described below, total dissolved solids are in the range 30.6–31.2 $g\, l^{-1}$. The transition metal content of the final