Cellular calcium pathways and isotope fractionation in

Emiliania huxleyi

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ABSTRACT

The marine calcifying algae Emiliania huxleyi (coccolithophores) was grown in laboratory cultures under varying conditions with respect to the environmental parameters of temperature and carbonate ion concentration \([CO_3^{2-}]\) concentration. The Ca isotope composition of E. huxleyi’s coccoliths reveals new insights into fractionation processes during biomineralization. The temperature-dependent Ca isotope fractionation resembles previous calibrations of inorganic and biogenic calcite and aragonite. Unlike inorganic precipitated calcite, the \([CO_3^{2-}]\) concentration of the medium has no significant effect on the Ca isotope composition of the coccoliths. These results indicate a decoupling of the chemical properties of the bulk medium and the calcifying vesicle. Cellular Ca pathways of E. huxleyi indicate that fractionation cannot occur at the crystal surface, as occurs during inorganic precipitation. The dominant processes leading to the observed Ca isotope fractionation pattern in E. huxleyi are most likely the dehydration of the Ca aquocomplex at the plasma membrane and the attachment of dissolved Ca to proteins of Ca channels. The independence of Ca isotope fractionation from \([CO_3^{2-}]\) and the small temperature dependence of E. huxleyi are also important for defining the isotopic signature of the oceanic Ca sink. Since coccolithophores contribute to about half the global CaCO3 production, a relatively uniform isotopic composition of the oceanic Ca sink is further supported.

Keywords: Emiliania huxleyi, calcium isotopes, coccolithophores, isotope fractionation.

INTRODUCTION

Coccolithophores are unicellular marine phytoplankton that surround themselves with small calcite plates called coccoliths. Their occurrence in large quantities in the upper surface ocean water makes them important primary producers responsible for approximately half of the global marine carbonate precipitation (Milliman, 1993). Hence, they play an important role for the carbon cycle (and CO2 balance) as well as for the marine Ca budget. Because their first occurrence dates back to the Triassic, they provide the potential to record long-term changes in ocean chemistry. For these reasons, their Sr/Ca ratios were studied in order to reconstruct growth and calcification rates (cf. Stoll and Schrag, 2000), while their Ca isotopic composition was used to reconstruct the Ca budget of the ocean (De La Rocha and DePaolo, 2000). Despite the important role of coccolithophores with regard to the carbon and calcium cycles in the ocean and proxy applications for paleoenvironmental reconstructions, trace-element and calcium transport as well as their calcification mechanisms are not fully understood.

Calcium isotope analyses might elucidate some of these processes in coccolithophores, because previous work indicates a strong kinetic effect of the carbonate ion concentration \([CO_3^{2-}]\) on calcium isotope fractionation (Lemarchand et al., 2004). Since reduced \([CO_3^{2-}]\) leads to a decrease in calcification rate of Emiliania huxleyi (Riebesell et al., 2000), it should also be reflected in the Ca isotopic composition of the coccolith. The apparent temperature dependence on Ca isotope fractionation was also proposed to be caused by \([CO_3^{2-}]\) via the temperature-dependent dissociation of carbonic acid (Lemarchand et al., 2004).

In this study, we investigate the main environmental parameters (temperature and \([CO_3^{2-}]\)) that affect Ca isotope fractionation for coccoliths. Based on these observations, we describe the Ca transport through the cell and the involved Ca isotope fractionation.

METHODS

Coccolithophore Culturing

We cultured the dominant coccolithophore Emiliania huxleyi (strain PML B92/11) in sterile filtered (0.2 µm) natural seawater (enriched with 100 µmol L\(^{-1}\) nitrate and 6.25 µmol L\(^{-1}\) phosphate, and with trace metals and vitamins similar to the description of Guillard and Ryther [1962]) at different temperatures between 5 and 20 °C (Fig. 1A). In addition, we cultured E. huxleyi at varying \([CO_3^{2-}]\), between 88 and 600 µM (at variable pH and constant dissolved inorganic carbon [DIC]), at constant temperature (20 °C). Cells were grown in dilute batch cultures with low cell densities and were harvested during the exponential growth phase; in all experiments, less than 5% of the DIC was consumed. The carbonate chemistry of the solution did not change significantly throughout the experiments and between parallel cultures. Coccolithophore culturing and carbonate chemistry analyses followed the descriptions of Zondervan et al. (2002) and Langer et al. (2006). Less than 1% of the dissolved Ca\(^{2+}\) was precipitated; therefore, changes of the Ca isotope composition of the fluid due to removal of light Ca isotopes, as observed in clathrites by Teichert et al. (2005), can be neglected for our experiments.

Sample Preparation and Ca Isotope Analysis

Prior to Ca isotope analysis, coccolith samples were bleached in a sodium-hypochlorite solution (~1% active chlorine) to remove organic components. Afterwards, the samples were washed in ultrapure water (pH 8–9 using added NH\(_4\)OH to prevent dissolution), in methanol (CH\(_3\)OH), and finally six times in ultrapure water. The \(^{44}\text{Ca}/^{40}\text{Ca}\) ratio of the coccolith CaCO\(_3\) was measured using thermal ionization mass spectrometry (TIMS) double-spike technique at the Leibniz Institute of Marine Sciences (IFM-GEOMAR), Kiel (Heuser et al., 2002). Isotope values are reported as 1000 ln \(\alpha\) [where \(\alpha = (^{44}\text{Ca}^{40}\text{Ca}_{\text{solid}})/(^{44}\text{Ca}^{40}\text{Ca}_{\text{fluid}})\)] as well as 10^6 ln(\(\alpha_{\text{mu}}\)) providing the isotope fractionation per 1 atomic mass unit (amu) (with \(\alpha_{\text{mu}} = e^{0.268\cdot x}\); Gussone et
RESULTS

Calcium Isotopes in Coccolith of *E. huxleyi*

The temperature dependence of Ca isotope fractionation in *E. huxleyi* is 0.027 ± 0.006‰/°C (Fig. 1A), similar to the planktic foraminifera *Orbulina universa* (0.019 ± 0.003‰/°C) (Gussone et al., 2003), but considerably smaller than the planktonic foraminifera *Globigerinoides sacculifer* (0.24‰/°C) (Nügler et al., 2000), which allowed the use of Ca isotopes as a temperature proxy (Zhu and Macdougall, 1998; Gussone et al., 2004). The Ca isotope fractionation observed for *E. huxleyi* was in good agreement with a previously published value of cultured *E. huxleyi* at 16 °C (1000·lnα = −1.3‰) and several carbonate oozes (De La Rocha and DePaolo, 2000). In contrast, Quaternary coccolith oozes of Zhu and Macdougall (1998) showed considerably lighter values (1000·lnα = −1.9 % to −2.6 %). The calcium isotope fractionation of cultured *E. huxleyi* samples that were processed without bleaching (open triangles in Fig. 1A), representing the total Ca in the cells (bound to organic compounds and carbonate), was identical to the fractionation of the bleached pure CaCO₃ samples.

Similar to *O. universa*, we observed only a minor dependence of Ca isotope fractionation in *E. huxleyi* on changes in the carbonate chemistry (Fig. 1B), which is in contrast to the strong [CO₃²⁻] dependence observed for inorganic calcite precipitation (Lemarchand et al., 2004).

DISCUSSION

Calcium Isotope Fractionation Mechanisms

Our culturing experiments show that *E. huxleyi* exhibits a small but significant temperature-dependent Ca isotope fractionation. For other marine species, as well as inorganically precipitated carbonates, Lemarchand et al. (2004) suggested that the small temperature dependence can be explained by the temperature-dependent speciation of the carbonic acid (Millero, 1995), leading to an increase in [CO₃²⁻] with increasing temperature. The increase in [CO₃²⁻] then leads to a higher saturation state of calcite and to increasing precipitation rates, resulting in reduced Ca isotope fractionation in the calcite.

While the observed temperature-dependent Ca isotope fractionation in *E. huxleyi* appears to be in agreement with the model by Lemarchand et al. (2004), we observed no significant dependence of Ca isotope fractionation on [CO₃²⁻]. This might imply that the observation that pCO₂ alters coccolith morphology and calcification rate (Riebesell et al., 2000), does not simply reflect the correlation between the extracellular and coccolith vesicle calcite saturation state. This finding is coherent with more recent data of Schulz et al. (2004), which show that under zinc limitation, the detrimental effect of changes in the carbonate system is significantly attenuated. This indicates that there is a more subtle biochemical system regulating the extent of calcification and coccolith morphology.

Further support for this reasoning is provided by the observation that coccolith production is a highly regulated process, in which the coccolith morphology strongly depends on the functioning of coccolith-associated polysaccharides (Henriksen et al., 2004; Young et al., 1999).

It is reasonable to assume a decoupling of the coccolith vesicle chemistry from the bulk solution because the ionic composition of the vesicle is defined by transmembrane ion transport; e.g., HCO₃⁻, H⁺, and Ca²⁺. A decoupling of the carbonate chemistry in the coccolith vesicle and the bulk solution is also required because carbon is transported into the vesicle as HCO₃⁻ (due to a pH of 7–7.2 in the cytoplasm; Brownelee and Taylor, 2004; Dixon et al., 1989), while H⁺, which is generated during CaCO₃ formation, has to be removed from the vesicle to keep the pH value inside the vesicle favorable for carbonate precipitation. In principle, it might be possible that the temperature-dependent speciation of the carbonate system (i.e., between CO₂, HCO₃⁻, and CO₃²⁻) inside the coccolith vesicle is responsible for the observed temperature-dependent Ca isotope fractionation, but a closer look at the Ca budget in *E. huxleyi* reveals that Ca isotope fractionation cannot occur during CaCO₃ precipitation inside the coccolith vesicle.

Cellular Ca Transport

The proposed Ca pathways in *E. huxleyi* are visualized in Figure 2: Ca is present in the bulk solution ([Ca²⁺] = 10 mM) as Ca²⁺.
or the Golgi apparatus. (3) Ca is transported into the coccolith vesicle, which is a concentration typically found in the plasma membrane, in endomembranes, and in the membrane of the coccolith vesicle, are similarly built (Evans and Williams, 1998), using the same basic Ca-binding mechanism, and are thus very likely to introduce the same isotope fractionation to the transported Ca. In addition, the Ca isotopic composition of the coccolithophore cell (including organically bound Ca in the soft tissue) exhibits identical values as the coccoliths (only pure CaCO 3; Fig. 1A), indicating that no fractionation occurs between soft tissue and the CaCO 3 of the coccolith. Therefore, Ca isotope fractionation most likely takes place at the plasma membrane, when Ca aq is dehydrated and attached to proteins at the entrance of a Ca channel.

Marriott et al. (2004) explained Ca isotope fractionation during calcite precipitation by equilibrium fractionation effects due to a decrease in bond strength and subsequent tendency to enrich the light isotope in the carbonate lattice. In contrast to inorganic precipitation, coccolithophores introduce a spatial separation between the site of dehydration and precipitation. Due to their internal Ca budget and Ca transport system, they prevent Ca isotope fractionation from occurring at the site of precipitation. The Ca isotope fractionation at the Ca channel might be thermodynamically comparable to the fractionation mechanism proposed by Marriott et al. (2004) for inorganic precipitation, since dehydration of Ca aq and attachment of Ca to the crystal surface might energetically resemble attachment of Ca to proteins, considering the similar Ca isotope fractionation and temperature sensitivities of inorganically precipitated calcite and coccoliths. This biologically induced isotope fractionation is expected to decrease with increasing temperature (a common behavior of stable isotope fractionation), leading to the observed positive correlation between Ca isotope fractionation and temperature.

The different Ca isotope fractionation of coccolithophores and foraminifera (Fig. 1) are most likely related to the different mechanisms involved in the Ca uptake. While in *E. huxleyi*, Ca isotope fractionation is mainly controlled by the dehydration of Ca aq at the plasma membrane, additional mechanisms have to be considered in foraminifera, like Ca uptake by endocyte of seawater vesicles, Rayleigh fractionation effects in cellular Ca reservoirs, and mixing of different Ca reservoirs.

CONCLUSIONS

The link between Ca isotope fractionation and cellular Ca pathways in *E. huxleyi* improves our understanding of proxy formation during coccolith biomineralization. The limited Ca reservoir in the coccolith vesicle, preventing Ca isotope fractionation at the site of precipitation, should be considered for the incorporation of trace elements (e.g., Sr) as well. By comparing Ca isotope fractionation of different species or materials, we find that similar temperature dependencies do not necessarily point to the same fractionation mechanisms. Our results have further implications for the application of Ca isotopes as an environmental proxy: The main factor influencing Ca isotopes in coccoliths is the isotopic composition of the seawater. The second parameter that affects Ca isotope fractionation is temperature, with a small
sensitivity of 0.027%/°C. Changes in ambient [CO₂] or pCO₂ introduce only a small variation in Ca isoform composition of coccoliths, which can be neglected for paleoceanographic reconstruction purposes. The result that the isotope fractionation between the coccolith calcite and the seawater is relatively uniform and is not strongly affected by temperature or CO₂ is of particular importance for Ca budget calculations, since coccolithophores are an important marine Ca sink, contributing about half of the marine carbonate production.

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