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Controls over $\delta^{44/40}$Ca and Sr/Ca variations in coccoliths: New perspectives from laboratory cultures and cellular models

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ABSTRACT

Coccoliths comprise a major fraction of the global carbonate sink. Therefore, changes in coccolithophores' Ca isotopic fractionation could affect seawater Ca isotopic composition, affecting interpretations of the global Ca cycle and related changes in seawater chemistry and climate. Despite this, a quantitative interpretation of coccolith Ca isotopic fractionation and a clear understanding of the mechanisms driving it are not yet available. Here, we address this gap in knowledge by developing a simple model (CaSri–Co) to track coccolith Ca isotopic fractionation during cellular Ca uptake and allocation to calcification. We then apply it to published and new $\delta^{44/40}$Ca and Sr/Ca data of cultured coccolithophores of the species *Emiliania huxleyi* and *Gephyrocapsa oceanica*. We identify changes in calcification rates, Ca retention efficiency and solvation–desolvation rates as major drivers of the Ca isotopic fractionation and Sr/Ca variations observed in cultures. Higher calcification rates, higher Ca retention efficiencies and lower solvation–desolvation rates increase both coccolith Ca isotopic fractionation and Sr/Ca. Coccolith Ca isotopic fractionation is most sensitive to changes in solvation–desolvation rates. Changes in Ca retention efficiency may be a major driver of coccolith Sr/Ca variations in cultures. We suggest that substantial changes in the water structure strength caused by past changes in temperature could have induced significant changes in coccolithophores' Ca isotopic fractionation, potentially having some influence on seawater Ca isotopic composition. We also suggest a potential effect on Ca isotopic fractionation via modification of the solvation environment through cellular exudates, a hypothesis that remains to be tested.

1. Introduction

Coccolithophores are marine unicellular calcifying phytoplankton, responsible for a large fraction of the $CaCO_3$ export to the deep ocean. Although their contribution to global primary productivity is small compared to other phytoplankton, coccolithophores are key modulators of the carbon cycle, accounting for ~50% of the $CaCO_3$ sediment production in the modern ocean (Milliman, 1993) and as much as ~95% of the pelagic $CaCO_3$ in the earliest Cenozoic. Variability in coccolithophores' contribution to $CaCO_3$ burial or their Ca isotopic fractionation could have the potential to impact seawater Ca isotopic composition ($\delta^{44/40}$Ca) over time, affecting the interpretation of the $\delta^{44/40}$Ca record in terms of the global Ca cycle (Fantle, 2010). The study of coccolith $\delta^{44/40}$Ca may provide important information not only about the past Ca cycle, but also about the physiological response of coccolithophores regarding Ca uptake and allocation to the calcification process under changing ocean chemistry and environmental conditions.

Culture experiments under variable temperature, carbonate chemistry, light, salinity and Ca and Mg concentrations have documented variation in Ca isotopic fractionation in coccolithophores (Gussone et al., 2007, 2006; Langer et al., 2007; Müller et al., 2011), which could translate into variations in Ca isotopic fractionation of this part of the carbonate sink. Gussone et al. (2006)

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proposed the first conceptual model of Ca isotopic fractionation in coccolithophores and suggested Ca dehydration during uptake across the plasma membrane to be responsible for the observed Ca isotopic fractionation. This Ca dehydration concept is equivalent to the more widely-used concept of desolvation, i.e. the removal of the solvent (in this case water) from the solute (in this case Ca) (Camacho et al., 2000). Desolvation is a key process controlling solute (e.g. proteins) transmembrane transport (e.g. Burton et al., 1992; Conradi et al., 1991). Although fractionation during desolvation has not yet been evaluated experimentally, molecular dynamic calculations have simulated a maximum fractionation of Ca isotopes during desolvation of −4.7‰ in solutions at infinite dilution, given the higher reactivity of the light calcium isotope (^{40}Ca) (Hofmann et al., 2012).

To identify the processes responsible for Ca isotopic fractionation in coccolithophores, here we implement the modeled potential for Ca isotopic fractionation during desolvation, in a new steady-state mass balance geochemical model of cellular Ca uptake and allocation to calcification in coccolithophores (CaSri–Co). We apply the model to interpret new coccolith $\delta^{44/40}$Ca and Sr/Ca results from *Emiliania huxleyi* and *Gephyrocapsa oceanica* cultured at varying CO_2 concentrations, as well as cultured *E. huxleyi* from previous studies (Gussone et al., 2006; Langer et al., 2007; Müller et al., 2011). The model elucidates how calcification rates, Ca retention efficiency, and the solvation environment properties control Ca isotopic fractionation and Sr/Ca in coccolithophores.

2. Materials and methods

2.1. Culture of coccolithophores

Coccolithophores of the species *E. huxleyi* (strain RCC 1216), *C. leptoporus* (strain RCC 1169) and *G. oceanica* (strain RCC 1292) were grown at Universidad de Oviedo at 18 °C on a 16/8 h light/dark cycle. Natural seawater for culturing was retrieved in the Cantabrian Sea during sampling campaigns in open waters 20 km from the coast. Seawater was enriched in nutrients according to the K/5 recipe (Keller et al., 1987), which contains no additional Ca or Sr. Cultures were acclimated to the experimental conditions for at least 6 generations, maintained in suspension by a continuous rolling system, and harvested at low cell density (<1.6 µg POC/mL) (Table 1). In pH manipulations, seawater carbonate chemistry was modified with the addition of 0.5 mol/L NaOH/HCl. In an additional experiment for *E. huxleyi*, seawater was acidified to remove dissolved inorganic carbon (DIC), and subsequently $NaHCO_3$ was added to adjust DIC to three different concentrations. None of the described methods for culture manipulation are expected to have affected the Ca concentration nor the Ca isotopic composition of the seawater used for culturing.

Total alkalinity (TA), pH, cell counts and growth rates were measured following standard procedures (Trimborn et al., 2009). The carbonate system was determined using CO_2 sys (Lewis and Wallace, 1998), applying equilibrium constants from Mehrbach et al. (1973) refitted by Dickson and Millero (1987). Carbon isotopic composition (δ^{13}C) of the media was analyzed at the time of cell harvest based on a continuous flow method similar to that described by Spötl (2005) using a gas preparation system GasPrep coupled to a Nu Instruments Horizon IRMS.

Cells were harvested for particulate organic carbon (POC) analysis on pre-combusted GF/F or QF/F filters and for particulate inorganic carbon (PIC) analysis on polycarbonate filters. PIC was determined by Ca yield using simultaneous dual inductively coupled plasma-atomic emission spectroscopy (ICP-AES, Thermo ICAP DUO 6300), following acidification with 2% HNO_3, except for *C. leptoporus*, where high Ca levels in blank filters precluded reliable determination of PIC. Ca depletion in the media remained <0.2% (Table 1), suggesting no significant changes of solution $\delta^{44/40}$Ca. Applying Rayleigh fractionation (Zeebe and Wolf-Gladrow, 2001), this maximum 0.2% in Ca depletion implies a maximum bias of 0.003‰ in our Ca isotopic fractionations, a value that is well below the error of measurements.

Cellular carbon quotas (organic carbon/cell, POC) were measured by flash combustion Elemental Analyzer (Euro Vector EA-1108) at 1020 °C coupled with a gas source isotope ratio mass spectrometer (Nu Instruments). Cell diameter and surface area are derived from cellular POC quotas using the regressions of Popp et al. (1998) since cellular carbon quotas scale predictably with biovolume. To compare calcification and Ca uptake among cells of different sizes, calcification rates were normalized to cell surface area.

δ^{13}C analyses were conducted to determine if there was any correlation between carbon and calcium isotopic fractionation. For $\delta^{44/40}$Ca and δ^{13}C analyses, harvesting was conducted on polycarbonate filters and cells were rinsed with high purity (Milli-Q) deionized distilled water and centrifuged to eliminate salt residues. For $\delta^{44/40}$Ca analysis, organic matter was oxidized following the method used in Stoll et al. (2012), with multiple steps of hot alkaline H_2O_2 solution. For δ^{13}C analysis, oxidation of organic matter was conducted following the method of Bairbakhish et al. (1999). Coccolith δ^{13}C was measured on a Nu Instruments Perspective dual-inlet isotope ratio mass spectrometer (DI-IRMS) connected to an automated carbonate preparation system (NuCarb) at the Universidad de Oviedo. Mean reproducibility of δ^{13}C was 0.05‰ (1σ). Carbon isotopic fractionation between the media DIC and the coccolith calcite ($\varepsilon_{coccolith}$) was calculated using the measured seawater media δ^{13}C of DIC at the end of the experiments. We use the term ε to refer to isotopic fractionation of *carbon*, while the term Δ is used to refer to isotopic fractionation of *calcium*.

2.2. Sample preparation for coccolith $\delta^{44/40}$Ca and Sr/Ca analysis

Coccolith calcite (~3–5 mg) was gently dissolved in weak 0.4 M acetic acid (~15–20 min). Splits of the $CaCO_3$ solution were used to determine Ca concentration and Sr/Ca ratios via dual inductively coupled plasma-atomic emission spectroscopy (ICP-AES) (Thermo ICAP DUO 6300) calibrated using the intensity ratio method, as described in Mejía et al. (2014).

For coccolith $\delta^{44/40}$Ca analysis, 120 µL of ^{43}Ca–^{48}Ca double spike was mixed with an aliquot of sample containing 3000 ng of Ca in pre-cleaned teflon beakers. After evaporation of the solution at ~100 °C, samples were reconstituted in 2.2 N HCl prior to analysis. For samples in which interfering compounds were affecting the isotope analysis due to isobaric interferences (e.g. $^{40}K^{+}$, $^{24}Mg^{19}F^{+}$, $^{25}Mg^{19}F^{+}$, $^{48}Ti^{+}$), Ca was purified and separated from other elements using column chemistry as previously described in Griffith et al. (2008).

2.3. Coccolith $\delta^{44/40}$Ca analysis

An aliquot of ~300 ng Ca loaded on an outgassed zone-refined Re filament was used for coccolith $\delta^{44/40}$Ca determination on a Finnigan Triton T1 thermal ionization mass spectrometer (TIMS) at the Helmholtz-Zentrum für Ozeanforschung Kiel, GEOMAR, closely following the method and use of standards described by Heuser et al. (2002). Tantalum chloride ($TaCl_5$) was used as activator to enhance Ca ionization efficiency. An in-house calcium fluorite (CaF_2) standard was included to monitor accuracy. Sample and standard $\delta^{44/40}$Ca values are referenced to the standard SRM 915a. External reproducibility assessed by repeatedly measuring CaF_2 was 0.17‰ (2σ, $n = 33$) and an average isotopic value of 1.45‰ was calculated, which is in the range of the mean CaF_2 isotopic value reported by Heuser et al. (2002) for multicup measurements