Available online at www.sciencedirect.com

ScienceDirect

Geochimica et Cosmochimica Acta xxx (2018) xxx–xxx

Geochimica et Cosmochimica Acta

www.elsevier.com/locate/gca

Carbonic anhydrase, coral calcification and a new model of stable isotope vital effects

Sang Chen [a,*], Alexander C. Gagnon [b], Jess F. Adkins [a]

[a] Division of Geological and Planetary Sciences, California Institute of Technology, Pasadena, CA 91125, United States
[b] The School of Oceanography, University of Washington, Seattle, WA 98195-7940, United States

Received 13 June 2017; accepted in revised form 16 February 2018; available online xxxx

Abstract

The stable isotope compositions of biogenic carbonates have been used for paleoceanographic and paleoclimatic reconstructions for decades, and produced some of the most iconic records in the field. However, we still lack a fully mechanistic understanding of the stable isotope proxies, especially the biological overprint on the environmental signals termed "vital effects". A ubiquitous feature of stable isotope vital effects in marine calcifying organisms is a strong correlation between $\delta^{18}O$ and $\delta^{13}C$ in a range of values that are depleted from inorganic calcite/aragonite. Two mechanisms have been proposed to explain this correlation, one based on kinetic isotope effects during $CO_2(aq)$-HCO_3^- inter-conversion, the other based on equilibrium isotope exchange during pH dependent speciation of the dissolved inorganic carbon (DIC) pool. Neither mechanism explains all the stable isotope features observed in biogenic carbonates. Here we present a fully kinetic model of biomineralization and its isotope effects using deep-sea corals as a test organism. A key component of our model is the consideration of the enzyme carbonic anhydrase in catalyzing the $CO_2(aq)$-HCO_3^- inter-conversion reactions in the extracellular calcifying fluid (ECF). We find that the amount of carbonic anhydrase not only modulates the carbonate chemistry of the calcifying fluid, but also helps explain the slope of the $\delta^{18}O$-$\delta^{13}C$ correlation. Differences in CA activity in the biomineralization process can possibly explain the observed range of $\delta^{18}O$-$\delta^{13}C$ slopes in different calcifying organisms. A mechanistic understanding of stable isotope vital effects with numerical models can help us develop better paleoceanographic tracers.

Keywords: Stable isotope vital effects; $\delta^{18}O$-$\delta^{13}C$ slope; Kinetic isotope effects; Carbonic anhydrase; Deep-sea corals

1. INTRODUCTION

The oxygen isotope composition of biogenic carbonates has been established as a proxy for past climate change for decades, since Urey (1947) first worked out the theoretical bases of the ^{18}O thermometer. The proxy is based on the temperature-dependent equilibrium isotope fractionation between carbonates and the fluid from which they precipitated. The successful application of the ^{18}O thermometer in biogenic carbonates relies on the assumption of equilibrium isotope fractionation, or at least a constant offset from equilibrium for the same category of organisms. However, disequilibrium isotope effects between carbonate and water have been widely observed in both laboratory experiments and natural samples. In the first experimental demonstration of the applicability of the ^{18}O thermometer in carbonates, McCrea (1950) noticed the dependence of the isotopic composition of the precipitated calcite on the percentage of carbonate ion in the solution at a constant temperature and $\delta^{18}O$ of water. This carbonate ion effect on the oxygen isotope composition of carbonates was later explained by oxygen isotope partitioning between the dissolved inorganic carbon (DIC) species (Usdowski and Hoefs, 1993; Beck et al., 2005), and was suggested by Zeebe (1999) to cause

* Corresponding author.
E-mail address: scchen@caltech.edu (S. Chen).

https://doi.org/10.1016/j.gca.2018.02.032
0016-7037/

the non-equilibrium oxygen isotope fractionation observed in inorganic experiments (Kim and O'Neil, 1997) as well as cultured foraminifera (Spero et al., 1997). An insight from the interpretation of Zeebe (1999) is the necessity to incorporate all the DIC species into the solid phase to explain its isotopic composition, making the carbonate-water fractionation ($\alpha_{c\text{-}w}$) a function of pH of the solution in addition to temperature. Follow-up work on this subject also observed a decrease of $\alpha_{c\text{-}w}$ with increasing growth rate of calcite (Gabitov et al., 2012; Watkins et al., 2013) and aragonite (Gabitov, 2013), suggesting kinetic isotope effects (KIE) from ion adsorption and desorption at the solution-solid interface during carbonate precipitation. This growth rate effect can cause an offset of the isotopic composition of the solid from the DIC pool (Watkins et al., 2014).

In addition to the temperature and pH dependent thermodynamic fractionation and growth rate dependent kinetic fractionation, the isotope exchange between the DIC species and water brings another source of disequilibrium, due to the relatively slow rate of CO_2(aq)-HCO_3^- inter-conversion. Although the timescale for one CO_2(aq)-HCO_3^- inter-conversion cycle is on the order of a minute at seawater pH, many cycles are required to achieve a complete exchange of oxygen atoms between the DIC species and water to reach isotope equilibrium (McConnaughey, 1989b). As a result, the equilibration timescale for oxygen isotopes in the DIC pool (hours to days) is significantly longer than for carbon isotopes (seconds to minutes), especially at high pH when the CO_2(aq)-HCO_3^- inter-conversion approaches an irreversible reaction (McConnaughey, 1989b; Zeebe and Wolf-Gladrow, 2001). The oxygen isotope equilibration timescale is longer than most natural calcification processes, as well as many laboratory experiments, which could lead to a significant expression of KIEs during hydration and hydroxylation of CO_2 and subsequently in the resulting carbonates. Carbon isotopes also experience KIEs during DIC speciation, but to a smaller extent than oxygen. The KIEs of hydration/hydroxylation were proposed by McConnaughey (1989a,b) to explain the strong correlation between $\delta^{18}O$ and $\delta^{13}C$ observed in a variety of marine calcifying organisms growing under the same environmental conditions (Fig. 1). McConnaughey (1989a) also pointed out the role of photosymbionts on the carbon isotope composition of coral skeletons, making the $\delta^{13}C$ more enriched compared to non-symbiotic corals.

The KIE mechanism by McConnaughey (1989a,b) predicts a simple linear relation between $\delta^{18}O$ and $\delta^{13}C$ in biogenic carbonates with a particular slope. A challenge to this mechanism was raised from a study of deep-sea corals (*Desmophyllum dianthus*), which observed a break in the $\delta^{18}O$-$\delta^{13}C$ linear relation at the most isotopically depleted end (Adkins et al., 2003). The optically dense central bands in these deep-sea corals, called centers of calcification (COCs), have similar $\delta^{13}C$ to the most depleted values in the surrounding aragonite fibers, but are more depleted in $\delta^{18}O$ than the secondary aragonite. The unique composition of the COCs causes a kink in the $\delta^{18}O$-$\delta^{13}C$ relation (Fig. 1a), which led Adkins et al. (2003) to propose a different mechanism for the stable isotope vital effects (Fig. 2). This mechanism was based on the observed pH up-regulation in corals within their ECF by the enzyme Ca-ATPase (Al-Horani et al., 2003; Venn et al., 2011). As Ca-ATPase pumps Ca^{2+} into the ECF in exchange for two protons, the pH of the ECF is raised, which shifts the DIC speciation toward the ^{18}O-depleted carbonate ion. Simultaneously, decreasing CO_2(aq) concentration in the ECF causes a larger ^{13}C-depleted CO_2 flux from the calicoblastic cells into the ECF, as opposed to ^{13}C-enriched DIC from the seawater leak. The simultaneous depletion of $\delta^{18}O$ and $\delta^{13}C$ during pH up-regulation stops when the pH of ECF reaches a threshold (pK_{a2}). After pK_{a2}, CO_2(aq) in the ECF is so low that the cell CO_2 flux is maximized, while the DIC speciation keeps moving from bicarbonate to carbonate ion, promoting further depletion of $\delta^{18}O$ in the $CaCO_3$. This is hypothesized to explain the $\delta^{18}O$-$\delta^{13}C$ kink produced by the COCs. In this model, however, Adkins et al. (2003) assumed equilibrium isotope fractionation between the DIC species. The neglect of KIEs during oxygen isotope exchange caused their numerical model to have a steeper $\delta^{18}O$-$\delta^{13}C$ slope than the observed range in deep-sea corals (1.9–2.6). In addition, the isotopic composition of the skeleton predicted by the composition of the DIC pool is offset from the expected inorganic aragonite values. This begs a new model of biomineralization to explain the observed $\delta^{18}O$-$\delta^{13}C$ data that takes into account the KIEs during DIC speciation, and applies the correct fractionation between the carbonate, DIC species and water.

Here we present a numerical model of coral calcification modified from Adkins et al. (2003). The fundamental components of the model are based on biological and geochemical observations of the coral calcification process, most of which were also considered in McConnaughey's seminal work (McConnaughey, 1989a,b). As observed in calcein and trace metal labeling experiments, seawater is directly involved in the coral calcification process (Gagnon et al., 2012; Tambutté et al., 2012). In our model, we treat ambient seawater as the starting material of the ECF, the composition of which the corals constantly modify. Corals actively up-regulate the pH of their ECF relative to ambient seawater, to locally increase saturation state and calcification rate, as suggested by direct pH measurements (e.g. Al-Horani et al., 2003; Venn et al., 2011) and boron isotopes (e.g. McCulloch et al., 2012; Wall et al., 2015). Ca-ATPase, an enzyme that exchanges protons for calcium ions, plays an important role in the pH up-regulation, and has been localized in the calicoblastic cells (Zoccola et al., 2004). In addition, our model considers the role of the enzyme carbonic anhydrase (CA) in the coral calcification process. CA is a ubiquitous enzyme found in almost all living organisms that catalyze the CO_2-HCO_3^- inter-conversion (Bertucci et al., 2013). The role of CA in the calcification process of corals has been suggested from CA-inhibition experiments for decades (Goreau, 1959), but it was only in the last decade that CA has been immunolocalized in the coral ECF and skeleton (Tambutté et al., 2007; Moya et al., 2008; Mass et al., 2014). Although these studies have only been performed on a few coral species, the presence of CA in the calcifying space of both symbiotic (*Stylophora pistillata*) and azooxanthellate (*Tubastrea aurea*)

Please cite this article in press as: Chen S., et al. Carbonic anhydrase, coral calcification and a new model of stable isotope vital effects. *Geochim. Cosmochim. Acta* (2018), https://doi.org/10.1016/j.gca.2018.02.032