Isotopic tracer evidence for the amorphous calcium carbonate to calcite transformation by dissolution-reprecipitation

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Abstract

Observations that some biogenic and sedimentary calcites grow from amorphous calcium carbonate (ACC) raise the question of how this mineralization process influences composition. However, the detailed pathway and geochemical consequences of the ACC to calcite transformation are poorly constrained. This experimental study investigated the formation of calcite from ACC by using magnesium and calcium stable isotope labeling to directly probe the transformation pathway and controls on composition. Four processes were considered: dissolution-reprecipitation, solid state transformation, and combinations of these end-members. To distinguish between these scenarios, ACC was synthesized from natural isotope abundance solutions and subsequently transferred to spiked solutions that were enriched in ^{43}Ca and ^{25}Mg for the transformation to calcite. Isotope measurements by NanoSIMS determined the $^{43}\text{Ca}/^{40}\text{Ca}$, and $^{25}\text{Mg}/^{24}\text{Mg}$ ratios of the resulting calcite crystals.

Analysis of the data shows the transformation is best explained by a dissolution-reprecipitation process. We find that when a small amount of ACC is transferred, the isotopic signals in the resulting calcite are largely replaced by the composition of the surrounding spiked solution. When larger amounts of ACC are transferred, calcite compositions reflect a mixture between the ACC and initial solution end-member. Comparisons of the measurements to the predictions of a simple mixing model indicate that calcite compositions 1) are sensitive to relative amounts of ACC and the surrounding solution reservoir and 2) are primarily governed by the conditions at the time of ACC transformation rather than the initial ACC formation. Shifts in calcite composition over the duration of the transformation period reflect the progressive evolution of the local solution conditions. This dependence suggests the extent to which there is water available would change the end point composition on the mixing line. While these findings have significant geochemical implications, the question remains whether this transformation pathway is generally followed when biomineralization involves ACC or is particular to these inorganic experiments. Insights from this study nonetheless suggest that some types of compositional variability, such as ‘vital effects’, may be explained in-part by a co-evolution of reservoir and products over the duration of the transformation.

1. INTRODUCTION

Reports that diverse phyla accumulate reservoirs of amorphous calcium carbonate prior to mineralization (Addadi et al., 2003; Addadi et al., 2012) are motivating an extensive effort to establish a process-based understanding of calcification via this pathway. Until recently, most ACC studies have focused on the structural aspects of ACC formation and transformation. The sea urchin provides a good example of this complexity with spicule growth starting as ACC that quickly transforms to calcite. Similar pathways involving ACC have been proposed in other organisms including snails (Hasse et al., 2000), bivalves (Baronnet et al., 2008; Jacob et al., 2011), crabs (Luquet and Marin, 2004; Dillaman et al., 2005), lobsters (Al-Sawalmih et al., 2009), isopods (Roer and Dillaman, 1984; Ziegler, 1994; Hild et al., 2008), earthworms (Gago-Duport et al., 2008; Lee et al., 2008), and sponges (Aizenberg et al., 1996; Sethmann et al., 2006),.

One crucial unanswered question is whether the chemical and isotopic signatures recorded in an initial ACC phase are transferred to the final calcite product. If so, this would suggest that many carbonate biomineral-based geochemical proxies could be controlled by ACC formation and subsequent transformation. The question of when and where chemical signatures are recorded during calcification by an ACC intermediate has particular significance for efforts to reconstruct paleoenvironmental conditions from the composition and isotopic signals contained in biominerals. Such correlations provide estimates of past chemical and physical parameters such as ocean pH (Vengosh et al., 1991; Hemming and Hanson, 1992; Yu et al., 2007), temperature (Epstein et al., 1953; Emiliani, 1954; Nürnberg et al., 1996; Lea et al., 1999), redox potential (Hastings et al.,

1996; Tribovillard et al., 2006), and productivity (Klein et al., 1996; Frank et al., 2000; Chase et al., 2001; Tribovillard et al., 2006).

While these environmental proxies provide remarkable insights, they also show ongoing inconsistencies that reiterate the need to establish process-based models for the signals that are recorded. For example, there are enigmatic differences between the compositions of biominerals versus inorganic counterparts that are grown under similar environmental conditions. There are also differences between the compositions of calcified biominerals from similar species that co-existed in similar environments. In the latter case, these “vital effects,” may be due to species-specific physiological controls over mineralization. Given that multiple pathways to biomineral formation are possible, one explanation for vital effects may be that differences reflect the predominant mineralization process— such as when calcite forms by a classical step growth process (Teng et al., 1998) versus transformation from an initial amorphous phase (Weiner and Dove, 2003; Bentov and Erez, 2006; Wombacher et al., 2011; Wallace et al., 2012).

For example, calcite grown by classical ion-by-ion attachment would be expected to record the composition of the individual components that are incorporated at the crystal surface. In contrast, signals that result from a mineralization process involving ACC may reflect the conditions of formation for the initial amorphous phase or be modified during the transformation to calcite. Therefore, the transformation itself may act as a gatekeeper that determines biomineral composition and, by extension, the trends that are measured in proxies. To decipher this complexity, a process-based understanding of nonclassical mineralization is critically needed. From this mechanistic basis, a robust approach for reconstructing past environmental conditions may become possible.

Two end-member interpretations have emerged in ongoing discussions of the process by which ACC transforms to calcite. Using evidence from microscopic and spectroscopic studies of skeletal structure, the first proposes that ACC transforms by a solid state process that involves dehydration and the structural rearrangement of ACC within membrane-bound compartments into crystalline products (Beniash et al., 1999; Politi et al., 2008; Weiner and Addadi, 2011; Gal et al., 2013). By this process, one expects the elemental and isotopic signatures measured in the final calcite to record conditions at the time of ACC formation. At the other end of the transformation spectrum, *in vitro* evidence indicates that accumulated ACC transforms by dissolving and reprecipitating as calcite (Pontoni et al., 2003; Lee et al., 2007; Han and Aizenberg, 2008; Wolf et al., 2008; Bots et al., 2012; Hu et al., 2012; Gal et al., 2013; Ihli et al., 2013). In this case, the final calcite product records the conditions of the local environment at the time of transformation.

These end-member interpretations are complicated by recent *in situ* TEM observations of physical contact between ACC and the crystalline phase during transformation that indicate both processes may occur simultaneously (Nielsen et al., 2014). This suggests a third possibility for the transformation that involves a combination of the solid state and dissolution-reprecipitation processes. These previous studies provide important insights but a definitive physical picture is elusive. To our knowledge, there is no chemical evidence to directly discern which of these processes is predominant.

This study reports direct chemical evidence regarding the ACC to calcite transformation process which allows us to determine when and where isotopic signatures are recorded. We designed a set of experiments that produce ACC from solutions labeled

with stable isotopes of ^{43}Ca and ^{25}Mg , then monitored the exchange of ions between solution, ACC and calcite during the transformation process. The rate of the ACC to calcite transformation was qualitatively regulated by including experiments conducted in solutions with three ratios of Mg/Ca. Measurements of isotope distributions show the transformation involves extensive interaction with the local solution. Little of the original ACC isotopic signature is retained, particularly for the higher Mg experiments that have longer transformation times. For the conditions of these longer duration experiments, the signatures after transformation fall on a mixing line between the ACC and solution end-members. These findings indicate the transformation is dominated by a dissolution-reprecipitation process and suggest the signatures contained in the calcite crystals record the local solution conditions at the time of transformation.

2. MATERIALS AND METHODS

2.1. Synthesis of ACC and transformation to calcite

ACC was synthesized by a modified version of the widely used ammonium carbonate decomposition method (Gruzensky, 1967; Addadi and Weiner, 1985; Paquette and Reeder, 1995; Lemarchand et al., 2004; Han and Aizenberg, 2008; Wang et al., 2009; Saulnier et al., 2012; Wang et al., 2012). Polystyrene well plates were used to conduct the experiments. Each well served as a ‘reactor’ and contained one glass coverslip (1.4 cm diameter), which provided a substrate for ACC precipitation and 3 ml of solution.

The initial solutions contained 10 mM calcium ($\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, Sigma Aldrich, >99%, dissolved in 18.2 M Ω -cm ultrapure water). To explore the influence of magnesium on ACC growth and transformation, the experimental design used initial

solution Mg/Ca ratios of 2.0, 5.0, or 6.0 (mol/mol) through the addition of a stock solution of 1.0 M Mg^{2+} ($\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, Sigma Aldrich, $\geq 99\%$). For this synthesis method, solution Mg/Ca drifts during precipitation due to reservoir (Rayleigh) processes, hence the reference to “initial Mg/Ca”. Supersaturation was achieved by adding 10 g $(\text{NH}_4)_2\text{CO}_3$ (Sigma Aldrich) to a separate and constantly stirred 20 mL volume of water in the glass jar. As NH_3 and CO_2 gases diffused from the $(\text{NH}_4)_2\text{CO}_3$ and into each well, the supersaturation increases as the pH rises to approximately 10 (Wang et al., 2012). The resulting ACC precipitated from the calcium and magnesium chloride solutions onto the glass coverslips and sides of wells, forming within minutes of the start of the reaction for all conditions.

The transformation of ACC to calcite was monitored using an optical microscope. Parallel experiments, where solids were removed at specific time points, were conducted to characterize the materials. For all experimental conditions, the ammonium carbonate source was removed before the transformation to calcite began. The onset of transformation varied from 2-5 hours to 16-24 hours for solutions with initial Mg/Ca ratios of 2.0 and 5.0-6.0, respectively. Upon complete transformation (about 5 days), the glass coverslips with calcite were removed, rinsed in ethanol, and dried in air. The crystalline products were identified as calcite or aragonite by Raman spectroscopy on a JY Horiba U1000 spectrometer (Wang et al., 2012). SEM images of the crystals (solution side) were acquired on a LEO (Zeiss) 1550 field emission scanning electron microscope under high vacuum and 5 kV accelerating voltage.

2.2. Isotope labeling using ^{43}Ca and ^{25}Mg

A subset of the wells was filled with isotopically labeled solutions. Enriched isotope spikes (Oak Ridge National Lab) were dissolved, and mixed with natural abundance reagents to achieve a simultaneous 3.8-fold enrichment of ^{43}Ca and 7.9-fold enrichment of ^{25}Mg for each solution. These large enrichments were chosen for two reasons: (1) they provide a unique label that is easily measured with NanoSIMS, and (2) for enrichments of several thousand permil, we assume natural fractionation effects are insignificant, where Mg and Ca fractionation between the solution and calcite during mineralization has an approximate magnitude of -4 to 0‰ (Galy et al., 2002; Chang et al., 2004; Immenhauser et al., 2010; DePaolo, 2011; Wombacher et al., 2011; Li et al., 2012; Saulnier et al., 2012; Mavromatis et al., 2014). This means that any fractionation that occurred during mineralization was too small to explain the isotopic signatures we measured in this study. The isotopic composition of the initial growth solutions (**Table 1**) were confirmed using an Elan DRC-II quadrupole inductively coupled plasma mass spectrometer (Q-ICP-MS; Perkin Elmer). Instrumental mass fractionation during Q-ICP-MS analysis was corrected using bracketing of enriched and natural abundance standards of known isotopic composition. The enriched isotope standard used in this part of the study was XG as described by Fernandez et al. (2010); the natural abundance standards were made from dissolved natural minerals

2.3. Experimental design

The experimental design used four wells run in parallel for each Mg/Ca solution ratio (2.0, 5.0 and 6.0 mol/mol) as shown in **Fig. 1A**. Two of the wells were designed to act as controls with the remaining two wells used during different stages of the

experimental treatment. The first control well contained natural abundance reagents during the entire experiment so that the ACC precipitated and transformed to calcite without artificially enriched isotopes (**Fig 1, first column**). A second control well was isotopically labeled. These experiments contained a solution enriched in both ^{43}Ca and ^{25}Mg isotopes for the entire experiment. Since the ACC in this control precipitated from an isotopically enriched solution and subsequently transformed to calcite in the same enriched solution, we would expect both the ACC and calcite to be isotopically enriched regardless of transformation pathway (**Fig 1, last column**).

The experimental treatment used two wells, one containing the same natural abundance solution as the natural abundance control, while the other well began with the same solution as the isotopically spiked control (**Fig 1, second and third columns**). Unlike the controls, where each glass coverslip was kept in the same solution during the whole experiment, coverslips were transferred between the different solutions after ACC precipitated but before the ACC transformed to calcite (**Fig 1B**). The timing of this transition was monitored using an optical microscope. The coverslip transferred from the natural abundance solution to the enriched solution was the focus for subsequent analysis in this study. In contrast, the coverslip that started in the enriched solution was removed before the ACC to calcite transition. The sole purpose of this coverslip was to ensure that the two wells had similar calcium and magnesium concentrations at the time of transfer.

The initial Mg/Ca solution conditions of 2.0 produced approximately half the amount of ACC on the glass coverslips as did the Mg/Ca conditions of 5.0 and 6.0. All coverslips were transferred to the isotopically enriched solutions for the transformation and returned to the glass jar with the ammonium carbonate source removed (**Section 2.1**).

These samples were allowed to transform for 5 days. A second set of experiments replicated the entire array of conditions (2.0, 5.0, or 6.0 Mg/Ca ratios and natural, spiked, or transferred ACC).

2.4. NanoSIMS spot analysis

The calcite products (e.g. Fig. 2AB) were analyzed at sub-micron spatial resolution using NanoSIMS, a surface analysis tool that can provide detailed measurements of elemental and isotopic composition. Samples were prepared for isotopic analysis by embedding calcite crystals from each experimental condition into indium metal. A 1 inch, 19-well sample holder was filled with melted In and, upon cooling, flattened using a hydraulic press at 10,000 psi for 10 seconds. For each condition, calcite crystals were transferred to the indium by pressing a coverslip upside down into an indium-filled well. This exposed their flat undersides for analysis (e.g. Figure 2CD and later discussion). All samples and standards were sputter-coated with a 30.5 nm thick gold film prior to analysis.

Isotope measurements were performed on the CAMECA NanoSIMS-50L (California Institute of Technology) using a duoplasmatron (O^+) source. The primary beam spot (measured as roughly 540 nm, full width and half max) was rastered during analysis to measure a well-defined square region with sides 2 microns in length. Total counts of ^{40}Ca , ^{43}Ca , ^{24}Mg , and ^{25}Mg were collected over 4.25 minutes for each spot analysis, including 75 seconds of presputtering to remove the gold coating followed by 60 cycles of 3 seconds duration each. The drift across cycles for each spot was consistent across the run and relatively reproducible with the standards. Single spot analyses were performed on 6-10 calcite crystals for each condition, identified during analysis using a

CCD camera and post-analysis using SEM for each experimental condition (for 81 total spots). Standards were measured regularly to bracket the samples, to correct for instrumental mass fractionation, and to establish accurate Mg/Ca. Additionally, a linear drift correction for instrumental mass fractionation was generated using all standards and natural abundance samples (assuming the solutions contained natural isotopic abundance). For all samples, the resulting crystals collected an approximately constant number of counts (within 20% and 5% relative error for ^{43}Ca and ^{25}Mg , respectively) across 60 measurement cycles (**Section 3.2**). This suggested a homogeneous depth distribution of the $^{43}\text{Ca}/^{40}\text{Ca}$ and $^{25}\text{Mg}/^{24}\text{Mg}$ within the calcite crystals.

The magnesium content of the calcite was measured by NanoSIMS by calibrating $^{24}\text{Mg}/^{40}\text{Ca}$ to % MgCO_3 of Mg-calcite and dolomite standards. The results from all sample conditions (natural, spiked, and transferred) were averaged across initial Mg/Ca solution ratios and fall within one standard error of previous %Mg estimates from [104] peak shifts on XRD (**Table 2**) (Wang et al., 2012). Three additional calcite standards were also measured throughout the NanoSIMS analysis to establish accurate Mg/Ca ratios, as described in Gabitov et al. (2013). The three calcite standards were Blue Calcite (Mg/Ca = 3.75 mmol/mol); UC Irvine Calcite (Mg/Ca = 3.446 mmol/mol); and Rensselaer Polytechnic Calcite (Mg/Ca = 0.396 mmol/mol), each of which have been shown to have Mg/Ca heterogeneity less than 3% (relative uncertainty) at the scale of SIMS analysis (Gabitov et al., 2013), and one dolomite standard (Mg/Ca = 1.00 mol/mol) to produce the calibration curve (**Fig. A.1**). The NanoSIMS was advantageous for this study over bulk chemical analysis because it enabled us to directly resolve the compositions of single

calcite crystals without the bias of the late stage aragonite that formed (Wang et al., 2012) and without the averaging effects of bulk analysis.

Following NanoSIMS analysis, SEM images of the embedded crystals were collected to identify spot locations and crystal integrity. By comparing the coordinates of the spot analysis to the ion beam marks in the SEM images (**Fig. 2CD**), we confirmed the polymorph associated with each spot analysis. This screening allowed us to reject data from crystals that were unidentifiable or identified as aragonite (14 spots). An additional 5 spots were rejected because they had low counts, which were attributed to rough topography or missing crystals. After data filtering, a total of 62 spot measurements remained with 3 to 10 spots for each experimental condition. It was not possible to determine the order of crystal formation within a given sample.

3. RESULTS

3.1. ACC precipitation and transformation to Mg-calcite

For all experiments, the ACC precipitated within minutes of adding the ammonium carbonate source. Its subsequent transformation into calcite slowed with increasing Mg content of the solution as reported previously (Folk and Land, 1975; Reddy and Nancollas, 1976; Loste et al., 2003; Wang et al., 2009; Wang et al., 2012; Xu et al., 2013). For experiments conducted in the lowest initial solution Mg/Ca ratio of 2.0, the transformation began after two hours to produce crystals with an elongated morphology reported previously (Han and Aizenberg, 2003; Loste et al., 2003) (**Fig 2AC**) and contained 19 ± 10 mol% MgCO_3 (**Table 2**). For experiments conducted in the 5.0 and 6.0 Mg/Ca solutions, a larger amount of ACC precipitate was produced on the coverslips and the transformation began at 16 to 24 hours. The resulting calcites grew

with characteristic spherical to botryoidal macroscopic morphologies (**Fig 2BD**) as reported by Wang et al. (2012). Composition analyses showed the 5.0 and 6.0 Mg/Ca solutions produced calcites that contained 41 ± 15 and 51 ± 17 mol% MgCO_3 , respectively (**Table 2**). The complete transformation of ACC to calcite in some experiments required up to 5 days.

Under all conditions, the transformation was followed by the nucleation and growth of secondary aragonite as confirmed by Raman and XRD analyses. This polymorph grew with an acicular morphology as individual spindles or as overgrowths onto the high-Mg calcite. This observation is consistent with previous studies using this method (Raz et al., 2000; Wang et al., 2012).

3.2. Isotopic analysis of controls and treatments

In the control experiments, natural abundance solutions resulted in natural abundance calcite crystals, while enriched solutions produced enriched calcite crystals, as expected. In greater detail, the raw number of ^{43}Ca and ^{25}Mg counts collected for a typical spot of a natural abundance calcite (**Fig 3A**) were significantly lower than calcites from a spiked experiment (**Fig 3B**). To evaluate the data collected for each spot analysis, we determined the isotope ratios ($^{43}\text{Ca}/^{40}\text{Ca}$ and $^{25}\text{Mg}/^{24}\text{Mg}$) for each of 60 measurement cycles as illustrated in **Fig 3C**, then averaged these ratios to obtain one set of mean $^{43}\text{Ca}/^{40}\text{Ca}$ and $^{25}\text{Mg}/^{24}\text{Mg}$ values for each crystal (**Table 3**). A similar process was used to produce average isotope ratios of each crystallite for all control and experimental treatments.

In a plot of measured $^{43}\text{Ca}/^{40}\text{Ca}$ vs. $^{25}\text{Mg}/^{24}\text{Mg}$ (**Fig. 4**), the natural abundance and spiked controls define two isotopically distinct end-members. First, the *natural abundance* controls have isotopic ratios that are clustered within a small range of values. This pattern is found for all initial Mg/Ca solution values (blue squares in **Fig 4ABC**). The *spiked controls* cluster in a region that is very distinct from the natural abundance controls (red triangles in **Fig 4ABC**). For the higher Mg/Ca experiments, the isotope ratio of the spiked control is more variable (**Fig 4BC**). Since few physically reasonable processes could result in this sort of fractionation, we assume this variability is caused by analytical issues. Indeed this variability could be related to the fact that only three viable measurements remained for the spiked controls for the Mg/Ca of 6.0 solutions after screening the data using the stated criteria (**Section 2.4**) and most of these crystals were intimately associated with late-stage aragonite. Even including all of these points, the variability is small compared to the isotopic difference between the natural abundance and enriched controls.

Recall the treatment experiments began by precipitating the ACC in natural abundance solutions and this material was transferred to the spiked solutions for the subsequent transformation to calcite (**Section 2.3**). Experiments that began in Mg/Ca of 2.0 produced calcite (five of the six crystals) that exhibit a narrow range of isotopic ratios (green circles in **Fig. 4A**) and have compositions similar to the spiked controls with the exception of one crystal (open green circle in **Fig 4A, Table 3**). In contrast, the higher Mg solutions produced calcite crystals with isotopic ratios that are intermediate between the natural abundance and spiked controls (**Fig 4BC, Table 3**). The 10 measurements from experiments that began with Mg/Ca of 5.0 solutions appear to show a mixing trend

which will be discussed in detail below. A similar trend is indicated by the Mg/Ca of 6.0 experiments (**Fig 4C**) for the three viable measurements. Sample number was limited because of extensive precipitation of late-stage aragonite.

4. DISCUSSION

4.1. Transformation pathways and mixing model

We use plots of $^{43}\text{Ca}/^{40}\text{Ca}$ vs. $^{25}\text{Mg}/^{24}\text{Mg}$ to determine which pathways are most important during the ACC to calcite transformation. Recalling that the treatment experiment produced ACC from a natural abundance solution (lower left end-member in **Fig. 4ABC**) and that this ACC was moved to an enriched solution for transformation (upper right end-member in **Fig. 4ABC**), then there are at least four possible transformation scenarios that can be distinguished from each other based on where the isotopic data from the treatment calcites plot relative to these end members. First, we consider that ACC transforms to calcite by a strictly solid state process, thus retaining the original natural abundance signature. In this case the resulting calcite would plot in the lower left corner together with the natural abundance control. None of the analyzed calcites from our treatment experiments are consistent with this pathway.

Second, the ACC transforms in the spiked solution by complete dissolution, but the amount of natural abundance ACC added to the solution during this dissolution process is small, resulting in little to no change to the solution isotope ratio. In this case, subsequent calcite reprecipitation would yield a solid that is similar to the isotopically

enriched control. This pathway can explain most of the data from the low Mg/Ca experiments (**Fig. 4A**), but not the other treatments.

The third and fourth transformation scenarios involve mixing between the two end-members. All four possibilities are illustrated in **Fig. 5**. The dashed curves (**Fig. 5**) were constructed assuming mixing between two end-members with different isotopic compositions and different Mg/Ca ratios using a simple binary hyperbolic function (**Appendix I, Table A.1**) (Vollmer, 1976; Langmuir et al., 1978). When the mixing relationships are plotted in $^{43}\text{Ca}/^{40}\text{Ca}$ vs. $^{25}\text{Mg}/^{24}\text{Mg}$ space, the end-member locations in **Fig. 5** depend upon isotope composition, but the curvature of the mixing relationship depends on the elemental composition of the end-members. For example, mixing with a high Mg/Ca end-member would tend to change the magnesium isotope ratio more rapidly relative to calcium isotopes than mixing with a low Mg/Ca end-member. Because ACC, calcite, and the solution in each well have very different Mg/Ca ratios, different mixing scenarios between these end-members will plot along lines with different curvature. Thus, while each of the mixing scenarios has process-specific concavity, the *degree of curvature* for both mixing trends is dependent upon the Mg/Ca ratio of an experiment.

The first mixing scenario we consider is a transformation process involving a combination of both solid state and dissolution-precipitation processes. This scenario would produce a concave up curve because the magnesium isotope signature of the solid mixture is dominated by the high Mg/Ca ACC end-member (**Fig. 5**). One way to visualize this transformation process is that some fraction of calcite is formed by an early stage solid state transformation and the remainder is produced by dissolution-reprecipitation as a later stage overgrowth from solution. This transformation process is

important to consider because it would likely result in the intimate association of crystallites whose size could be below the resolution of the NanoSIMS analysis. Comparing this scenario with our data, none of our experiments follow a concave-up mixing trajectory.

In the final scenario, we consider a situation where the composition of the solution evolves due to dissolution of ACC during a dissolution-reprecipitation pathway. This will occur if a large amount of ACC dissolves relative to the amount of solution that is present. In this scenario, the mixing curve would be concave down, because the solution has a higher Mg/Ca than ACC, with the magnesium isotope signature of the mixture dominated by the spiked solution end-member. During calcite precipitation, the crystal will capture the isotope ratio of the surrounding solution, thus the isotope ratios of different calcite crystals will plot along a mixing curve that reflects the evolving solution composition. The process is entirely dissolution-reprecipitation but the calcite is growing from a changing solution environment.

4.2 Qualitative insights for the transformation process

Returning to the experimental data in **Fig. 4ABC**, the measurements show a distinct concave down trend for all solution compositions, which suggests that the transformation is a predominantly dissolution-reprecipitation process (e.g. **Fig. 5**). There is no evidence for a solid state transformation within the resolution of these data.

For experiments that began with Mg/Ca = 2.0 (**Fig 4A**), the $^{25}\text{Mg}/^{24}\text{Mg}$ and $^{43}\text{Ca}/^{40}\text{Ca}$ values measured in the calcite products are clustered near and *to the left* of the composition of the spiked solutions where they transformed. This indicates extensive

dissolution-reprecipitation (e.g. discussion of **Fig 5**). The offset from the spiked end-member (control) is likely due to mixing with the natural abundance isotopes that are released during dissolution of the initial ACC. This shift is small because 1) the amount of ACC transferred is small (see **Section 2.2**) and 2) the complete transformation in these low Mg experiments occurred within a short period. The products thus present a cluster of similar, enriched compositions.

In contrast, the treatments that began with the higher solution Mg/Ca ratios (5.0, 6.0) produce calcite crystals with a span of isotopic ratios (**Fig 4BC**). These data exhibit a mixing trend with the distinct concave down curvature suggesting a transformation process that is dominated by dissolution and reprecipitation. The values in **Fig 4B** suggest good agreement with the predictions of the theoretical model (compare experimental measurements with dashed line).

To understand the physical basis for the span of measured isotopic ratios in **Fig 4BC**, recall the higher Mg solutions required a longer time for the complete transformation of ACC to calcite and produced twice the amount of ACC material on the coverslips. Thus, a greater mass of ACC relative to the solution volume was transferred into the well (**Section 2.2**), which introduced a larger mass of the natural abundance isotopes. Upon transformation in the spiked solutions, the dissolution of this larger quantity of material induces an increasingly greater shift in the isotope ratios of the solution over the course of the transformation to calcite. Thus, we suggest the span of compositions measured in these experiments is the compounded effect of 1) larger amounts of ACC on the coverslips transferred to the spike solution and 2) the

progressively greater shift in local solution composition during the longer time to transformation.

At the highest Mg/Ca solutions (**Fig 4BC**), the mixing model has the same curvature but a small and systematic offset from the experimental measurements. Recall the endpoint of the model is governed by the solution isotope composition (**Section 4.1**) and the spiked control measurements exhibited a higher variability than the natural control measurements (**Section 3.2**). Thus, we attribute the offset in the mixing model to analytical uncertainties in the isotopic compositions of the solution or the spiked controls. While this offset limits quantitative interpretation of the data, the mixing trend exhibited by the data supports the explanation that 1) the ACC to calcite transformation is a predominantly dissolution-reprecipitation process for the conditions of these experiments and 2) the values measured in the final calcite products record the local solution environment at the time of transformation.

4.3. Implications

For the conditions of this experimental study, the formation of calcite from an inorganic ACC intermediate occurs by a dissolution-reprecipitation process. The isotopic ratios contained in the initial ACC are largely replaced to produce calcites with signals that reflect the solution composition at the time of transformation. When the amount of ACC that precipitates is relatively large compared to the size of the solution reservoir, the calcite compositions plot on a mixing curve that record a mixture between dissolved ACC and the solution. Experiments that require a longer transformation period (through higher Mg/Ca ratios) are qualitatively associated with a greater spread of isotopic compositions. We interpret this trend as the temporal evolution of solution composition.

Our observations of the isotopic co-evolution of solution and solid imply a similar evolution in the elemental composition of the final products is also active during the transformation.

The findings could help us understand what controls biomineral compositions for situations where mineralization involves an intermediate phase. For example, a solid state transformation during biomineralization would lock the distinct geochemical signals associated with ACC into the final skeleton (ex. high Mg/Ca) such that the proxy behavior would be strongly influenced by the initial ACC composition. Our study, however, shows that when the transformation is dominated by dissolution-reprecipitation, the composition of the ACC can be completely erased or partially remodeled such that signals in the final calcite bear little resemblance to the initial conditions. While the geochemical implications of a dissolution-reprecipitation process are clear, the question remains whether this transformation process is generally followed during biomineralization or whether this process is particular to our inorganic experiments.

Insights from this study also raise the point that final mineral composition is influenced by the amount of ACC relative to the volume and concentration of elements in the calcifying solution. Under a dissolution process, compositional variations in the calcite products are greater when the amount of ACC transferred is large relative to the reservoir. This experimental observation leads us to ask how the size of a reservoir in a mineralizing biological system influences final skeletal composition and implies a complex sensitivity to local reservoir conditions. Such a dependence suggests the extent to which there is water available would change the end point composition on the mixing line. Insights to this question are found in Gal et al. (2014), which indicates the

457 reprecipitation process remains active to much lower mineral/water ratios than those used
458 in our study. For example, our experiments used 3 mL of solution in the well plates.
459 While we did not measure the total mass of ACC that formed, we can use the calcium and
460 carbonate ion concentrations to estimate the maximum production of ACC per well was 3
461 mg. Thus, our system is approximately 3 mg ACC/3 mL solution. A closer look at the
462 Gal et al. data show the dissolution-reprecipitation process continues to be active when 3
463 mg of ACC transforms in only 0.2 mL water (15X less water). Given these, admittedly
464 semi-quantitative comparisons, it is interesting to ask if there is a transition in the
465 transformation process with progressively lower water content.

466 Our findings that the isotopic signature varies with the time to transformation,
467 presumably due to Mg content, may have the potential to explain some aspects of proxy
468 variability that occur in synthetic crystals and biominerals. For example, uncertainties
469 that are presently interpreted as random noise, experimental error, or vital effects, may
470 reflect a co-evolution of reservoir and products over the duration of the transformation.

471 Finally, it is intriguing to consider the reverse question of whether the skeletal
472 products of carbonate-producing organisms contain compositional trends that can inform
473 the biomineralization pathways. One may be able to use isotopic signatures to
474 reconstruct pathways when combined with additional information about an organism. By
475 integrating insights from multiple pathways to mineralization with the host of
476 environmental, chemical and biological factors that influence growth, a robust physical
477 basis for interpreting and predicting biomineral compositions may become possible.

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Fig. 1: Experimental design for ^{43}Ca and ^{25}Mg isotope labeling of solutions and solid phases. Four wells were filled with solutions that contained a natural abundance of isotopes (blue solutions, columns 1 and 2) or an enriched isotope abundance (red solutions, columns 3 and 4). Solids forming from the well plate in column 1 correspond to the natural abundance control. In contrast, column 4 corresponds to a control where the solid is isotopically labeled. The two well plates in the columns 2 and 3 were used for the experimental treatment. (A) At time = 0, a glass coverslip was placed in each well and ACC was allowed to precipitate after adding the carbonate source. (B) Before the onset of transformation to calcite, which occurred at 2-5 hours (for $\text{Mg}/\text{Ca} = 2.0$) or 16-24 hours ($\text{Mg}/\text{Ca} = 5.0$ and 6.0), the glass coverslip in the enriched well (column 3) is removed and replaced by the coverslip from the natural isotope abundance well. (C) After five days, the ACC completely transformed. Collectively this experimental design produces three types of samples for analysis: the natural and spiked controls and the transferred (natural to spiked) treatments.

A. $t = 0$ hrs

B. $t = 2$ or 16 hrs

C. $t = 5$ days

Fig. 2: SEM images of representative calcite morphologies. (A and C) initial Mg/Ca = 2.0 produces calcite with an elongated morphology; (B and D) initial Mg/Ca = 5.0 produces spherical to botryoidal calcite. Crystals pressed into indium present a flat underside (C and D) suitable for NanoSIMS analysis. The white dashed circle indicates the area of a darker “V” shape, which represents the beam damage around each $\sim 2 \mu\text{m}^2$ analysis area. Scale bars = 20 μm .

Fig. 3: Typical NanoSIMS spot analysis show the typical isotope counts collected over 60 cycles of measurements for two high-Mg calcite crystals (initial Mg/Ca = 5.0) (A) Natural abundance control. (B) Spiked control shows higher ^{25}Mg and ^{43}Ca counts for calcites that transformed from ACC in a spiked solution. (C) Calculated isotope ratios for the natural and spiked controls in A and B. The spiked calcite control shows ^{43}Ca and ^{25}Mg enrichments that are consistent with solution isotope abundance (compare to **Table 1**).

A. Natural control

B. Spiked control

C. Ratios for natural and spiked controls

Fig. 4: The isotope ratios determined from calcites for the natural abundance controls (blue squares), the spiked controls (red triangles), and experimental treatments that began as ACC in natural abundance solutions and were transferred to spiked solutions for transformation to calcite (green circles). Three solution compositions were used: (A) $\text{Mg}/\text{Ca} = 2.0$, (B) $\text{Mg}/\text{Ca} = 5.0$, and (C) $\text{Mg}/\text{Ca} = 6.0$. Predictions of mixing model for each solution composition are shown as a dashed line (Section 4.1).

Fig. 5: Predictions of mixing model show isotope-specific signatures expected for calcite that transforms from ACC: (1) complete solid state rearrangement; (2) complete dissolution-reprecipitation; (3) combination of solid state transformation and partial overgrowth of calcite by reprecipitation; and (4) combination of dissolution-reprecipitation where the progressive dissolution of ACC affects solution composition. Comparisons to the experimental measurements (**Fig 4ABC**) indicate the transformation occurs by dissolution-reprecipitation. Mixing lines in this image are calculated for solutions with $\text{Mg}/\text{Ca}=5.0$.

Table 1: Summary of solution isotope enrichments. The $^{43}\text{Ca}/^{40}\text{Ca}$ and $^{25}\text{Mg}/^{24}\text{Mg}$ of natural and spiked solutions are obtained from Q-ICP-MS analysis.

Initial Mg/Ca ratio	$^{43}\text{Ca}/^{40}\text{Ca}$			$^{25}\text{Mg}/^{24}\text{Mg}$		
	Natural	Spiked	Enrichment	Natural	Spiked	Enrichment
2.0	0.00138	0.00516	3.8-fold	0.129	1.012	7.9-fold
5.0	0.00138	0.00454	3.3-fold	0.129	1.005	7.8-fold
6.0	0.00138	0.00510	3.7-fold	0.129	1.022	7.9-fold

Table 2: The Mg content (mol% MgCO_3) of calcite averaged across all isotope conditions (natural, spiked, and transferred to spike calcites) is obtained from NanoSIMS and compared to XRD estimates from previous work using the same growth conditions. Errors are reported as one standard deviation of all crystals analyzed.

Initial Mg/Ca ratio of solution	Mg content (mol% MgCO_3)	Mg content (mol% MgCO_3) ^a
2.0	19±10	12.2±0.2
5.0	41±15	35.2±0.8
6.0	51±17	36.6±1.3

^a(Wang et al., 2012)

595 **Table 3:** Summary of isotope ratio measurements used in Fig. 4.
 596

	$^{43}\text{Ca}/^{40}\text{Ca}$	$^{25}\text{Mg}/^{24}\text{Mg}$
Mg/Ca = 2.0		
Natural controls	0.001420	0.1262
	0.001403	0.1305
	0.001359	0.1301
	0.001387	0.1310
	0.001351	0.1271
	0.001374	0.1294
	0.001322	0.1285
	0.001333	0.1281
	0.001422	0.1275
	0.001400	0.1286
Spike controls	0.005696	0.9523
	0.005209	0.9895
	0.005646	0.9343
	0.005756	0.9424
	0.005645	0.9240
	0.005848	0.9454
	0.005764	0.9374
Transferred samples	0.001521	0.4580
	0.004691	0.9893
	0.004524	0.9800
	0.004535	1.0422
	0.004628	1.0419
Mg/Ca = 5.0		
Natural controls	0.001393	0.1255
	0.001349	0.1288
	0.001337	0.1286
	0.001268	0.1291
	0.001525	0.1256
	0.001245	0.1356
Spike controls	0.005852	1.0541
	0.005667	1.0693
	0.006247	1.0783
	0.006040	1.0801
	0.005611	1.1429
	0.005799	1.2312
	0.005609	1.1696
	0.005619	1.1841
	0.005628	1.1869
Transferred samples	0.003311	0.8820
	0.003295	0.8290
	0.001815	0.4522
	0.002387	0.6027
	0.001753	0.5496
	0.002037	0.6851
	0.002834	0.9327
	0.003232	0.8796
	0.002682	0.8865
	0.001903	0.5079
Mg/Ca = 6.0		
Natural controls	0.001320	0.1313
	0.001233	0.1348

	0.001373	0.1340
	0.001480	0.1236
	0.001481	0.1252
	0.001438	0.1189
	0.001265	0.1234
	0.001460	0.1279
	0.001448	0.1343
Spike controls	0.004150	1.0752
	0.006402	1.2553
	0.004180	1.0788
Transferred samples	0.002382	0.8101
	0.002676	0.9231
	0.001667	0.5962

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