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# Formation of ordered dolomite in anaerobic photosynthetic biofilms

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## ABSTRACT

Dolomite enabled the preservation of fine microbial textures in some Archean and Proterozoic marine microbialites, but has rarely done so during the Phanerozoic. Here, we report precipitation of dolomite in anoxygenic photosynthetic biofilms grown under chemical conditions relevant for Archean seawater. Ordered dolomite nucleates primarily on the surfaces of photosynthetic cells when manganese(II) is also present, nanocrystals of disordered dolomite form on exopolymeric substances (EPS) in microbial cultures grown either in the dark or without manganese. Dolomite nucleation and maturation on different surfaces in photosynthetically active cultures amended with 0.1-1 mM manganese(II) enables the preservation of biofilm textures at scales larger than individual microbial cells. This provides a new model for the preservation of microbial textures by dolomite before the oxygenation of the oceanic photic zone.

## INTRODUCTION

Microbes, exopolymeric substances (EPS) and organic surfaces can mediate the nucleation of protodolomite in some shallow hypersaline and supratidal, evaporation-prone environments (Bontognali et al., 2010; Sanchez-Roman et al., 2008; Shinn et al., 1965) and deep organic-rich sediments colonized by methanogenic Archaea and sulfate reducing bacteria (Kenward et al., 2009; Krause et al., 2012). High-Mg phases that form in this manner typically does not preserve the primary textures of photosynthetic microbial mats or fine ooid laminae (Bontognali et al., 2010; Spadafora et al., 2009). Thus, these models do not adequately account for the origin of dolomite with a high content of manganese or iron that preserved fine microbial textures in some Archean and Proterozoic microbialites (e.g., Veizer et al., 1990; Wright, 2000; Wright and Tucker, 1990).

Here, we use experiments to investigate the preservation potential of benthic photosynthetic communities under chemical conditions relevant for carbonate-depositing marine environments during the Archean and the Proterozoic Eons. Benthic communities before the rise of atmospheric oxygen are thought to have relied on sulfide, Fe(II), Mn(II) or hydrogen as electron donors, and the oceans contained ~0.1 mM of iron and manganese (Anbar and Holland, 1992; Beukes, 1987), although more recent estimates suggest lower iron concentrations in the deep ocean (Eroglu et al., 2018). This motivates our use of microbial communities driven by sulfide-based photosynthesis in low-sulfate and 0.1-1 mM manganese solutions that are in equilibrium with a high $pCO_2$. The culture medium contained less than 0.9 μM sulfate, 8 μM Fe(II) and less than 4 nM $O_2$ (Pajusalu et al., 2018). The medium was amended by 0.05 mM $H_2S$ and 0.1-1 mM $MnCl_2$, and was in equilibrium with an atmosphere of 80% $N_2$ and 15% $CO_2$ at pH 7. We describe the mineral composition, nucleation and maturation as a function of the medium composition, microbial activity and types of microbial surfaces. The detection of ordered dolomite as the primary precipitate in our cultures inspires a new model for the formation of early, fabric-retentive high-Mg carbonate phases in manganese-rich sediments.

## METHODS

Samples of sediments were retrieved from Fayetteville Green Lake (FGL), New York, by a metallic gravity scoop and photosynthetic biofilms were enriched from these samples. All batch enrichment cultures were grown anaerobically in modified basal FGL (MFGL) medium for two weeks (supplementary information section 1 in the GSA Data Repository[1]). The medium was reduced by the addition of 20-50 μM sulfide. Sulfide was also the main electron donor for photosynthesis. The composition of microbial communities in enrichment cultures was analyzed by high-throughput Illumina sequencing. The biofilms did not contain abundant sulfate reducing bacteria. All photosynthesizing cultures, dark culture controls, and sterile controls (without microbes) were incubated for two weeks or longer. The medium was supersaturated with respect to the precipitation of both calcite ($SI_{Calcite}$= 0.85) and dolomite ($SI_{dolomite}$= 2.42) (Supplementary data section 5), but sterile controls did not contain precipitates. Precipitates from all microbial cultures were characterized by X-ray powder diffraction (XRD), scanning electron microscope (SEM) and transmission electron microscopy (TEM) (supplementary information section 2, 3 and 4). Each experiment was performed at least three times.

## RESULTS

The green sulfur bacterium *Chlorobium limicola* was the most abundant microbe and the only photosynthetic organism in the red-brown biofilms. Various anaerobic non-photosynthetic bacteria such as *Geobacter* sp. and *Acholeplasma* sp. were also present (Daye et al., submitted). *Chlorobium* sp. that was incubated in the presence of light was photosynthetically active, and approximately 0.5-1 mm thick biofilms grew in two weeks. The pH in the active cultures increased from 7.24 to 7.33 (0.6%) and the alkalinity decreased from 3958.3 mg/L to 3858.5 mg/L (2%) (DR Section 6).

Sterile controls did not contain any detectable precipitates after two weeks, but an ordered dolomite phase was abundant in photosynthetic cultures amended with $MnCl_2$ (Fig. 1; Fig. DR2; Table DR1). This mineral was identified by the $2\Theta° = 30.9$ peak corresponding to the basal reflections of (104) crystal plane, the presence of superstructure reflections (hkl), and the match with the dolomite standard (Fig. DR2). The 101 ordering reflection was not visible, but 015, 113 and 021 ordering reflections were present (Gregg et al., 2015; Reeder, 1983) (Fig. DR2, FigDR3A, B). Dolomite in these cultures encrusted the surfaces of cells (Figure 1, Fig. DR4 A-C) and nucleated on EPS (Fig. DR5). Calcium carbonate and elemental sulfur, which is the expected product of sulfide-based photosynthesis by Chlorobi, were the main precipitates in photosynthetic cultures that were not amended with $MnCl_2$ (Fig. DR2; Table DR1). Only small amounts (per ~ 10 mg of the analyzed biofilm) of disordered dolomite formed in the dark cultures amended with $MnCl_2$ or in photosynthesizing cultures that were not amended with $MnCl_2$ (Figure DR1, Table DR1, Fig. DR4D, DR4E). These minerals nucleated on EPS, and did not encrust cell surfaces (Figs. DR6, DR7). Dark cultures without Mn(II) did not contain any detectable minerals (Fig. DR8). Thus, anaerobic photosynthetic biofilms promoted the precipitation of ordered dolomite only in the presence of light and Mn(II).

Dolomite in photosynthesizing, Mn-amended cultures nucleated after one week as an amorphous Ca-Mg carbonate phase with < 5 nm domains (Fig. 1A-D). This phase nucleated on the surfaces of cells and contained manganese (Fig. 1B). After an additional week, the amorphous nuclei matured into < 5 nm rounded nanocrystals in polycrystalline aggregates. The more mature nanocrystals had a uniform lattice fringe and interplanar spacing of 2.19Å corresponding to (11-3) crystal plane of dolomite (Fig. 1E-H). Both the amorphous phase and the more mature

nanocrystals were detected primarily on the surfaces of *Chlorobium* sp. cells, recognized by both spinae (Fig. 1A, E), and chlorosomes, i.e., complexes of photosynthetic antennae (Fig. DR4 A-C). Some dolomite grains < 200 nm in diameter were also present on the fibrous EPS (Fig. DR5). Cell surfaces in photosynthesizing cultures that were incubated without Mn(II) were not encrusted by any minerals (Fig. DR4D, E), but < 200 nm wide dolomite grains in these cultures nucleated on EPS and matured into 1 μm aggregates (Fig. DR6). Calcite nucleated on EPS in dark cultures (Fig. DR7).

To further characterize the organic surfaces and microbial textures in anoxygenic photosynthetic biofilms, we imaged horizontal cryo-thin sections of a 400 μm thick biofilm by SEM/EDS (Fig. 2, Figs. DR9,10). The crystal sizes and the extent of mineral coverage depended both on the type and the depth (age) of organic surfaces. At 25 μm below the surface of the biofilm (Fig. 2A), dense aggregates of cells contained >2 μm wide aggregates of dolomite crystals. These crystals nucleated on cell surfaces, did not replicate the shapes of individual cells, and were distributed sporadically across the horizontal surface (Fig. 2B). Five to ten μm wide globular dolomite covered more extensive areas in cell-rich zones deeper in the biofilm (Fig. 2E, F) and in older biofilms (Figs. DR9,10). Spatially discontinuous minerals smaller than < 1 μm were directly associated with fibrous exopolymeric substances 50 μm below the surface of the two-week old biofilm (Fig. 2C, D). Thus, differences in the shape and size of dolomite crystals reflected the relative densities of cells and EPS. SEM imaging of two-month old biofilms confirmed the persistence of these trends over time (Figs. DR9, 10), and the dolomitizing biofilms did not exhibit evidence of extensive degradation or mineral dissolution. Rather, small minerals that nucleated on EPS matured into ~ 2 μm wide grains.

## DISCUSSION

Active anoxygenic photosynthetic microbes, exopolymeric substances and dissolved manganese stimulate the nucleation and early maturation of ordered dolomite. This process occurs in a well-buffered bulk medium and does not depend on large, metabolically induced changes in pH and alkalinity (Bosak and Newman, 2003), but requires the surfaces of photosynthetically active *Chlorobium* cells as well as EPS (Fig. 1, Figs. DR5-7). Our observations are consistent with a report of dolomite in the zones of some modern microbialites where manganese oxyhydroxydes are brought in close contact with intermediate and reduced sulfur species (Petrash et al., 2016). Differences between the biofilms of various sulfur-cycling or oxygen-producing photosynthetic microbes, such as purple or green sulfur bacteria, purple non-sulfur bacteria and cyanobacteria, are an open question.

Previous studies proposed roles for organic compounds in the desolvation of $Mg^{2+}$-water complexes on the growing surfaces of carbonate crystals to form dolomite (Roberts et al., 2013; Zhang et al., 2015). This process, mediated by EPS, carboxylic groups or dead cells under aerobic or anaerobic conditions, likely accounts for the nucleation of poorly crystalline, less ordered high-Mg calcite and protodolomite (Bontognali et al., 2014; Roberts et al., 2013). In contrast to many of these studies, we observe ordered dolomite. Given that the intensities of the 015 and 110 ordering reflections were not equal, the mineral formed under our experimental conditions was not perfectly ordered (Gregg et al., 2015). This can be attributed primarily to the incorporation of Mn(II) into the dolomite crystal structure. This process should cause the disappearance of the 101 ordering reflection and a shift toward larger d-values.

The mechanism by which manganese helps nucleate dolomite in systems where the concentration of magnesium exceeds that of calcium is currently not known. Previous studies

showed that Mn(II) inhibits the formation of synthetic dolomite at high temperatures (Lumsden et al., 1989). However, the addition of 0.1-1 mM Mn(II) markedly increased both the abundance and the ordering of dolomite in photosynthetic biofilms (Fig. 1B, F, Fig. DR2). This is consistent with the previously noted requirement for Mg(II) in the formation of high-Mn dolomite-type mineral phases in inorganic ternary systems of $CaCO_3$-$MnCO_3$-$MgCO_3$ carbonates (Mucci, 2004). The phases formed in these inorganic experiments did not contain more than 10% Mg (Mucci, 1988), but our observations suggest that the formation of authigenic high-Mg carbonate phases in Mn-rich anoxic, microbially colonized marine sediments is also possible.

Microbial textures preserved by microcrystalline dolomite or high-Mg calcite record information about microbial processes and interactions with sediments at the sediment-water interface and/or during early burial. Micrometer-size dolomite grains that form in anoxygenic photosynthetic biofilms, the expected products of their maturation/diagenesis, and the reduction of sediment porosity due to the presence of microbial mats may account for the exceptional preservation of textures in some Archean and Proterozoic Mn- or Fe-rich dolomitic microbialites, coated microbial filaments and grains. In all these instances, the fabric-retentive, Mn- or Fe-rich dolomite that contains organic inclusions is interpreted as a product of early diagenesis of a high-Mg precursor under anaerobic conditions (Simonson and Jarvis, 1993; Wright, 2000; Wright and Tucker, 1990). If high-Mg phases are already abundant at the sediment-water interface, different compositions of Ca and Mg isotopes can be expected (Blättler et al., 2015; Higgins et al., 2018). We hypothesize that fabric-retentive early dolomite that nucleates in benthic communities will appear sediment-buffered (*sensu* Higgins et al., 2018), perhaps due to the sealing properties of mineral-rich microbial mats. These predictions can be tested by studies of fabric-retentive dolomites.

The new model for microbial dolomite formation and the reports of well-preserved textures of Archean dolomitic microbialites inspire the following hypothesis: the distribution of texture-preserving dolomite in time and space may reflect the changing abundances of anoxygenic or oxygenic phototrophs as well as concentrations of reduced ions in the pore fluids due to the progressive oxygenation of the marine realm. For example, the abundant limestone in the shallower stromatolitic and oolitic strata of the large carbonate platform in the Neoarchean Ghaap Group of the Campbellrand-Malmani is consistent with the inferred redoxcline on the shelf of the C-M platform based on iron minerals and isotopes (Eroglu et al., 2018). By the same token, the fine dolomitic laminae from the older, Mesoarchean microbialites (Fig. 1, Bosak et al., 2013; Siahi et al., 2016) may record active anoxygenic photosynthesis in those benthic environments. These hypotheses can be explored by comparing the preservation potential of different microbial communities under a range of redox and other chemical conditions, the composition of minerals in these communities, as well as the associated calcium and magnesium isotope signatures.

## CONCLUSION

Nano- and microcrystalline ordered dolomite forms in anaerobic photosynthetic biofilms that use sulfide as the primary electron donor and grow in the presence of 0.1-1 mM Mn(II). Dolomite nucleates on the surfaces of microbial cells or EPS. In cell-rich areas, this mineral matures into microcrystalline globular minerals. In contrast, nanocrystalline minerals that nucleate on fibrous extracellular polymeric substances remain finer grained and preserve the porosity of EPS-rich areas. These observations provide a new model by which to account for the nucleation of Mn-rich dolomite in pore fluids and for the preservation of microbial textures by fabric-retentive dolomite during the Archean and Proterozoic.

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## Figure captions

**Figure 1.** Transmission electron micrographs (TEMs) of cells incubated in the light with 1 mM Mn(II) for two weeks. Darker areas in A and E are covered by minerals, long protuberances are *Chlorobium* sp. spinae. A: TEM at 200 kV of a *Chlorobium limicola* cell. Dotted rectangles mark the areas analyzed by EDS, high resolution TEM (HRTEM) and selected area X-ray diffraction (SAED). B: EDS spectrum of the area shown in A. C: HRTEM shows only an amorphous phase. D: Diffraction pattern of the same amorphous phase, scale bar: 5 1/nm. E: TEM at 200 kV of a cell with spinae. Dotted rectangle marks the area analyzed by EDS HRTEM and SAED. F: EDS spectrum of the area shown in E. G: HRTEM shows the lattice fringe of dolomite nanocrystals at the cell surface. H: Diffraction pattern corresponds to dolomite with (104) superstructure diffraction, scale bar: 5 1/nm.

**Figure 2.** Cryo-SEM of horizontal sections through a two-week old biofilm. A: Cell-rich area 25 µm below the surface. Bright granules are dolomite. The dashed rectangle shows the area magnified in B. B: White arrows point to bacterial cells; the two bright globules are dolomite. C: Porous EPS 50 µm below the surface. The dashed rectangle outlines the area magnified in D. D. EPS network with sporadically distributed brighter areas that contain minerals. E: Cell-rich area

80 µm below the surface. The area contains numerous dolomite grains. The dashed rectangle outlines the area magnified in F. F: White arrows point to bacterial cells; the very bright round aggregates are dolomite crystals.

[1]GSA Data Repository item 201Xxxx, [materials and methods for media and enrichment protocol, X-ray diffraction, scanning electron microscopy, transmission electron microscopy and saturation indices, as well as figures (DR1, DR2, DR3, DR4, DR5, DR6) and table DR1] is available online at www.geosociety.org/pubs/ft20XX.htm, or on request from editing@geosociety.org or Documents Secretary, GSA, P.O. Box 9140, Boulder, CO 80301, USA.