



Calcite Precipitation by Cyanobacteria

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Prokaryotic picoplankton plays an important role in lacustrine calcite precipitation, especially in oligotrophic lakes. The mechanisms of cyanobacteria-surface mediated precipitation reactions, however, remain poorly understood. For interpreting geochemical and isotopic information stored in sediments, it is essential to know the carbonate precipitation mechanisms, environmental and microscopical details of nucleation on the cell surface. A laboratory study was designed, investigating the environmental conditions in the process of calcite precipitation by cyanobacteria. This study was combined with electron and atomic force microscopy in order to obtain insights into the details of the calcite precipitation mechanisms.

Under carefully controlled chemical and physical conditions, precipitation of calcite was induced by adding cultures of cyanobacteria to supersaturated solutions of CaCO_3 ($\Omega = 2 - 20$) at different $\text{CaCl}_2/\text{NaHCO}_3$ ratios. The cell suspensions were purged with an artificial atmosphere at constant CO_2 concentration. Abiotic solutions were used as reference systems. In a series of bulk experiments induction times for the onset of precipitation were measured with and without cells. The morphology of the precipitates was then analyzed by scanning electron microscopy. Both initial crystals (~ 500 nm) and larger grown crystals (> 10 μm) showed rhombohedral and hexagonal-prism shapes which are characteristic for calcite.

In order to investigate the nucleation of calcite on the cell surface under controlled conditions, experiments with *Synechococcus* (PCC 7942) were performed in a flow-through design of an atomic force microscope (AFM). The AFM was operated in 'Tapping Mode' providing information on the topography and additionally on physicochemical properties by mapping the phase shift of the oscillating cantilever. For these experiments cells were immobilized on glass slides. As the basic requirement, growth

experiments were performed in order to ensure that the fixed cells were alive. The division of a single *Synechococcus* cell and the growth of cell agglomerates were monitored over a periods of several days.

In a second step, calcite nucleation experiments were performed. Under carefully controlled physical and chemical conditions, nucleation of calcite was enhanced by flushing the fluid cell of the AFM with supersaturated solutions of CaCO_3 . These solutions were 7 and 10 times supersaturated. As the result of the addition of the CaCO_3 -solution, changes of the microtopography and physicochemical properties of the cell surface were observed. These changes in microtopography – the formation of little bumps at the cell surface - could be proven by transmission electron microscopy of ultrathin sections of the cells prepared by ultramicrotomy.

Other sections ($\sim 100\text{nm}$) of cyanobacteria cells and attached calcite crystals were prepared by Focused Ion Beam. This technique allowed us to preserve the cell wall - crystal interface in order to perform TEM analysis. Using TEM-Electron Energy Loss Spectroscopy, carbon bonds in both calcite crystals and cyanobacteria cells were detected and identified. The carbon absorption spectra are significantly different to discriminate the organic and inorganic carbon. Thus, the absorption peaks are the fingerprints of the analyzed material and can be used to analyze the transition zone between the cell and the particle.

Thus this study reveals further insights into nucleation mechanisms of calcite on the surface of cyanobacteria cells at different spatial scales.