Calcium carbonate formation processes on cyanobacterial surface

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Cyanobacteria is oxygenic phototroph known to calcify under optimum ambient water chemistry. Fossils of calcified cyanobacteria are widely spread especially during the Phanerozoic, and the fluctuation in their abundance is crucial for reconstructing paleo-ocean chemistry (e.g., pH). Two major mechanisms are suggested for cyanobacterial calcification: 1) the photosynthetic increase in $CaCO_3$ saturation state, and 2) the crystal nucleation sites supplied by acidic groups of extracellular polymeric substances (EPS). Previous study based on natural samples demonstrated that acidic EPS is crucial for controlling the degree and the style of calcification. However, the influence of EPS on crystal morphology, crystal arrangement, and precursor (amorphous calcium carbonate: ACC) formation are still not well understood. The present study, therefore, examined the relationship between physical/chemical characteristics of EPS and calcification styles by using cultivated cyanobacteria.
Four cyanobacteria having different EPS characteristics (*Spirulina*, *Phormidium*, *Scytonema*, and *Leptolyngbya*) were cultivated. Acid-base titrations and lectin binding analysis indicated that all investigated cyanobacteria secreted EPS containing acidic groups. These cyanobacterial cultures were incubated in an aquarium (pH = ca. 8.5, [Ca^{2+}] = 2 mM, DIC = 2 mM) with light supply to cause calcification. Microelectrode measurements (pH, O_2, and Ca^{2+}) showed that all cyanobacterial cultures induced $CaCO_3$ precipitation by photosynthesis, although the degree of precipitation was different. Observations by transmission electron microscopy (TEM) and scanning transmission X-ray microscopy (STXM) revealed that the characteristics of precipitated $CaCO_3$ were significantly different among the investigated cyanobacteria. Especially, calcite formed around *Spirulina*, *Phormidium*, *Scytonema* exhibited specific features including ACC, sub-micron-sized calcite polycrystals, and superstructures, suggesting that the nucleation occurred on acidic EPS via ACC nanoparticles. In contrast, such features were rarely observed in calcite formed around *Leptolyngbya* producing only a few amounts of EPS. From these results, $CaCO_3$ formation processes on cyanobacterial surface is suggested as follows: 1) ACC nanoparticles are formed on acidic EPS, 2) crystallization takes place inside of grown ACC particles to form submicron-sized calcite polycrystals, and 3) these polycrystals grow into calcite single crystal while ACC and polycrystals remain partly.

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