Role of extracellular polymeric substances on cyanobacterial calcification

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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 supply 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. Especially for the calcification style, 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.
cyanobacteria having different EPS characteristics (*Spirulina, Leptolyngbya, Phormidium* and *Scytonema*) were cultivated, and the quantity and quality of acidic groups in their EPS were assessed by acid-base titrations and lectin binding analysis. These cyanobacterial cultures were incubated in an aquarium (pH = about 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. However, the degree of calcification and the morphology of resulting $CaCO_3$ crystals were significantly different: *Spirulina* lacking EPS was not calcified and crystals (about 1 mm diameter) were slightly scattered around, *Leptolyngbya* secreting non-acidic EPS was not calcified and rhombic crystals (about 10 mm diameter) scattered around, *Phormidium* secreting partially acidic EPS was externally calcified by cubic crystals (about 20 mm diameter), and *Scytonema* secreting acidic EPS was externally calcified by rhombic crystals (about 20 mm diameter). These results demonstrated that EPS has strong impact on cyanobacterial calcification. For *Scytonema*, an ultra-thin section was prepared by focused Ion beam processing and observed by transmission electron microscopy (TEM) and scanning transmission X-ray microscopy (STXM). Observations indicated that the mineral precipitated upon the EPS of *Scytonema* was mostly monocrystalline calcite, while features of ACC were recognized at the vicinity of EPS (about 100-200 nm thick). Spotty concentration of carboxylic groups at the outer margin of EPS suggests that such acidic regions would be the starting points of ACC precursor, which would subsequently form calcite nucleation.

