Tempered and self-limiting calcite formation directed by coccolith organic macromolecules†

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Coccoliths are mineralized organic scales that are formed inside the cells of coccolithophores, a dominant group of marine unicellular algae.‡ Coccolith formation occurs inside an intra-cellular vesicle, where calcite crystals nucleate and grow on the rim of an organic scale called base plate. Soluble macromolecules are an important component inside the coccolith vesicle, and become tightly associated with the mineral phase. A previous study, investigating the coccoliths of the species Pleurochrysis carterae, showed that macromolecular recognition between the soluble macromolecules, mainly the three known polysaccharides, and the rim of the base plate, directs large amounts of calcium ions to the site destined for crystal formation. Interestingly, a mineral phase was not observed, raising the question of how mineralization can be achieved in the system. In the current study, we report the formation of calcite within this Ca-rich environment at conditions where crystallization from the solution is absent. Crystalization in these conditions is strictly restricted to the privileged microenvironment controlled by the organic macromolecules, yielding oriented thin calcite crystals on the base plate.

P. carterae coccoliths were harvested from cultured cells, and their calcite crystals dissolved. The organic fraction, base plates as well as soluble macromolecules, was retained after dialysis. The base plates are oval in shape, 1–2 μm in diameter and ~10 nm thick, and are barely visible in a scanning electron microscope (SEM) on the supporting membrane (Fig. 1A). When calcium ions (1 mM) are added to the coccolith organic fraction, they trigger the macromolecular recognition reaction between the negatively charged soluble macromolecules and the rim of the base plates, as reported earlier. This reaction leads to the aggregation of calcium-loaded macromolecules at the rim of the base plate. This Ca-rich phase is not mineralized (Fig. 1B). In the present experimental procedure, aliquots of Na₂CO₃ solution were directly mixed into the reaction volume, following the formation of the Ca-rich phase on the coccolith base plate, with the aim of inducing mineral formation. For a wide range of carbonate concentrations the system behaved as expected for calcium carbonate precipitation in the presence of organic additives.

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At low concentrations of carbonate (less than 5 mM) no precipitation was observed, and at sufficiently high concentrations (more than 20 mM) uncontrolled precipitation of rhombohedral calcite occurred (Fig. 1C and Table S1, ESI†). A detailed investigation of the carbonate concentration range needed to bring the solution close to the conditions that yield precipitation revealed a concentration window in which a completely different behavior occurs (Table S1, ESI†). If carbonate is added to a concentration of 10 mM and the solution is left to ripen over the course of 4 hours, the distinctive rhombohedral calcite crystals, indicative for homogeneous nucleation and subsequent solution-mediated crystal growth, can no longer be detected. Instead, different objects of uniform size and shape are obtained (Fig. 1D). Higher-magnification micrographs show that these objects are base plates that are encrusted with a newly formed phase (Fig. 1E). A close inspection shows that only the upper, mineral-associated, side of the base plate is covered by this phase while the lower side is bare (Fig. 1F). Base plates with deposits of the new phase are a minor fraction of the total base plates present in the sample. The deposit-free base plates seem to have lost most of their initial ring composed of the un-mineralized calcium-rich phase (Fig. 1G).

We used high-angle annular dark-field scanning transmission electron microscope (HAADF-STEM) to characterize the encrusted base plates (Fig. 2A and Fig. S1, ESI†). High-magnification imaging reveals highly ordered lattice fringes that matched the crystal structure of calcite (Fig. 2B). Electron diffraction pattern from a single mineralized base plate shows a single set of diffraction spots (Fig. 2C), suggesting that the calcite is aligned. Atomic force microscope (AFM) images show that the mineral coverage of the base plate is 60–100 nm thick, with granular morphology of fused particles, 10–50 nm in diameter (Fig. 2D). The crystallographic registry of the calcified base plates was investigated using transmission Kikuchi diffraction (TKD) in SEM. Mapping the c-axis orientation throughout the mineralized base plate shows a narrow distribution around a single orientation (Fig. 2E and F). Interestingly, in all the analyzed base plates, with only one exception (9 out of 10), the c-axis orientation is ±25° deviating from the supporting film plane, and the a-axis perpendicular ±25° to it. Since the calcified base plate usually is not lying flat on the support, we attribute much of the orientation variation to the variability in sample positioning, and conclude that a common orientation of the calcite crystals relative to the base plate is observed: the c-axis lies in the base plate plane and the a-axis is vertical to it.

We investigated several aspects underlying this crystallization process, which leads to a thin single crystal of calcite. The most striking observation is that none of the mineralized base plates grew further than the ~100 nm thin calcite cover. This cannot be growth cessation due to depletion of calcium and/or carbonate, because adding a measured amount of carbonate resulted in the mineralization of more base plates with the same morphology rather than further growth of the existing crystals (Fig. 3A and B).

Quenching the reaction after 10 minutes showed that initial mineral precipitation occurs on the base plate rim in the form of an amorphous phase containing calcium and carbonate.

Fig. 1  (A) SEM image of isolated base plates. The inset shows intact P. carterae coccoliths, with their baseplates indicated with asterisks. (B) When 1 mM calcium ions are added to the base plates and the soluble macromolecules, they form dense aggregates around the base plate periphery. (C) Adding 50 mM carbonate leads to the precipitation of rhombohedral calcite crystals. (D) However, if only 10 mM carbonate ions are added, calcite growth from solution is suppressed and only the formation of precipitates on base plates can be seen after few hours. (E and F) Higher magnification of base plates encrusted only on one side. (G) Two encrusted base plates, with opposite orientations, and many un-mineralized base plates (several are indicated with asterisks) that lost most of the Ca-loaded macromolecules at their rims. Note that for imaging with the SEM all samples were dried on a track-etched membrane. Thus, the 100 nm round holes that are visible in the images are part of the support, and not of the sample.
ions (Fig. 3C, D, Fig. S2 and S3, ESI†). Most likely, the crystallization proceeds from the circumference of the base plate inwards, and an amorphous precursor phase is an intermediate phase. The carbonate buffer system plays only an indirect role in this process as similar results are obtained at pH values from 8 to 11, as long as the carbonate concentration is appropriate (Table S1, ESI†). Increasing calcium concentration results in crystallization of rhombohedral crystals in addition to the base plates (Table S2, ESI†). All these observations suggest a mechanism in which crystallization is restricted only to the Ca-rich phase that is formed by the soluble macromolecules. To test this hypothesis, experiments where the soluble macromolecules were diluted with respect to the base plates were carried out. These experiments show that the restriction of the crystallization process to the base plate surface is gradually lost with the dilution, as the crystals grow outwards developing rhombohedral facets (Fig. 3E, F and Table S2, ESI†). This is in agreement with the Ca-rich phase losing its unique properties and becoming more similar to the surrounding solution.

The results of these in vitro experiments suggest that coccolith organic components can manipulate the chemical environment in their vicinity and funnel the crystallization process to a prescribed outcome via the following steps: adding calcium ions to the coccolith organic components triggers the macromolecular recognition reaction, and results in the formation of three distinct calcium environments (Fig. 4A and B). Some calcium ions are aggregated at the base plate periphery, another fraction is more loosely complexed by macromolecules that are still suspended in the solution, and some calcium ions are left unbound. Adding carbonate ions to this system leads to differential interactions with the different calcium environments (Fig. 4C). Since unbound calcium ions are abundant, if the solution saturation level is high enough, solution-mediated crystal nucleation and growth will prevail. However, we experimentally found that there is a carbonate concentration range that is not high enough to induce crystallization in the solution, yet sufficient to induce precipitation in the Ca-loaded aggregates at the base plate periphery (Fig. 4D). This crystallization process starts by heterogeneous nucleation on the base plate surface. Due to a kinetic barrier, not all the base plates nucleate crystals, and the ones that do not lose their associated calcium...
Fig. 4 The different components and their interactions during the base plate crystallization experiments. Base plates and soluble macromolecules (represented as coils) are in black. Calcium, either as ions or dense phases, is in red. Carbonate is depicted in blue, and calcium carbonate in purple. The insets show one base plate in a detailed view.

in a process that resembles Ostwald ripening. What is evident by the current observations is that propagation of crystal growth is restricted to the nanoscale Ca-rich phase created by the interaction of the macromolecules and calcium ions. How such dense chemical environment facilitates crystal nucleation and growth, and what chemical interactions occur between the organic and inorganic components, need to be further elucidated.

Controlling the stochastic process of crystallization in solution is highly challenging. The investigated in vitro crystallization process, using macromolecules extracted from coccoliths, points to a potentially important biological strategy. Negatively charged polymers complex calcium ions, and by means of specific attachment to a compatible surface, a microenvironment is created that is the preferred site for crystallization upon addition of the counter-ion. Such a process can inspire engineered nano-patterning of inorganic solids that is not based on the delicate balance of thermodynamic considerations of solution-mediated crystal growth, but rather on the specific interactions between polymers, recruiting the inorganic material to prescribed locations.

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Notes and references