Title

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The potential of biomineralization for Carbon Capture and Storage

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Summary

Employment of cyanobacteria in biomineralization of carbon dioxide by calcium carbonate precipitation offers novel and self-sustaining strategies for point-source carbon capture and sequestration. Although details of this process remain to be elucidated, a carbon-concentrating mechanism, and chemical reactions in exopolysaccharide or proteinaceous surface layers are assumed to be of crucial importance. Cyanobacteria can utilize solar energy through photosynthesis to convert carbon dioxide to recalcitrant calcium carbonate. Calcium can be derived from sources such as gypsum or industrial brine. A better understanding of the biochemical and genetic mechanisms that carry out and regulate cynaobacterial biomineralization should put us in a position where we can further optimize these steps by exploiting the powerful techniques of genetic engineering, directed evolution, and biomimetics.

Introduction

Strategies to reduce emissions of carbon dioxide (CO_2) from fossil fuels, and hence mitigate climate change, include energy savings, development of renewable biofuels, and carbon capture and storage (CCS). For CCS, several scenarios are being considered. One approach is capture of point-source CO_2 from power plants or other industrial sources and subsequent injection of the concentrated CO_2 underground or into the ocean [1]. An alternative to this point-source CCS method is expansion of biological carbon sequestration of atmospheric CO_2 by measures such as reforestation, changes in land use practices, increased carbon allocation to underground biomass, production of biochar, and enhanced biomineralization [2]. In addition to geological or oceanic CO_2 injection, novel models for point-source CCS based on accelerated weathering and biomineralization are emerging, utilizing either abiotic [3-5] or biotic [4,6,7] processes.

Biomineralization of CO_2 by calcium carbonate ($CaCO_3$) precipitation is a common phenomenon in marine, freshwater, and terrestrial ecosystems and is a fundamental process in the global carbon cycle [8].

Precipitation of $CaCO_3$ can proceed by either or both the following reactions:

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$Ca^{2+} + 2HCO_3^- \leftrightarrows CaCO_3 + CO_2 + H_2O$ (1)

$Ca^{2+} + CO_3^{2-} \leftrightarrows CaCO_3$ (2)

with reaction 2 being the principal path, at least in seawater [9,10].

Bicarbonate (HCO_3^-) is ubiquitous in water and is formed via dissolution of gaseous CO_2:

$CO_{2\,(aq)} + H_2O \leftrightarrows H_2CO_3$ (3)

$H_2CO_3 \leftrightarrows HCO_3^- + H^+$ (4)

The concentration of carbonic acid (H_2CO_3) is small so the dissolved CO_2 from reactions 3 and 4 occurs predominantly as HCO_3^-.

A fraction of HCO_3^- dissociates to form carbonate (CO_3^-):

$HCO_3^- \leftrightarrows H^+ + CO_3^{2-}$ (5)

The lion's share of global calcification takes place through biotic processes in the oceans. Although the oceans are supersaturated with Ca^{2+} and CO_3^-, spontaneous precipitation of $CaCO_3$ in the absence of calcifying (micro)organisms is rare owing to various kinetic barriers [11]. The contribution of microorganisms, particularly cyanobacteria, in $CaCO_3$ precipitation and sedimentation is substantial and it has played a major role in geological formations since the Archaean Era [12]. Although studies of microbially mediated biomineralization through $CaCO_3$ precipitation have a long history, the mechanistic details of the different steps are only poorly understood [13]. In this review we discuss the potential for microorganisms, specifically cyanobacteria, in calcification, that is conversion of CO_2 to recalcitrant calcium $CaCO_3$.

We begin our discussion on cyanobacterial calcification and its potential in CCS by a brief description of the general features of cyanobacteria where we elaborate on the carbon -ncentrating mechanism (CCM) that allows cyanobacteria to actively take up inorganic carbon (C_i) from the external medium and perform efficient photosynthesis in aqueous environments. We then give an account on microbial biomineralization, specifically as it occurs in cyanobacteria. In this context we return to the CCM and point out the intimate association between CCM and the calcification process. Finally, we ask how biomineralization by calcifying cyanobacteria can contribute to CCS, and we point out research areas that should be prioritized to tackle some of the challenges ahead.

Cyanobacteria

Cyanobacteria are photosynthetic Gram-negative bacteria that carry out oxygenic photosynthesis and are thought to be the origin of chloroplasts of plants and eukaryotic algae via endosymbiotic events in the late Proterozoic or early Cambrian period. Cyanobacteria occupy a wide array of terrestrial, marine, and freshwater habitats, including extreme environments such as hot springs, deserts, bare rocks, and permafrost zones. In their natural environments, some cyanobacteria are often exposed to the highest rates of UV irradiance known on our globe. Cyanobacteria also have an extensive fossil record. Indeed, the oldest known fossils are of cyanobacteria from Archaean rocks of western Australia, dated 3.5 billion years old. Through their photosynthetic capacity cyanobacteria have been tremendously important in shaping the course of evolution and

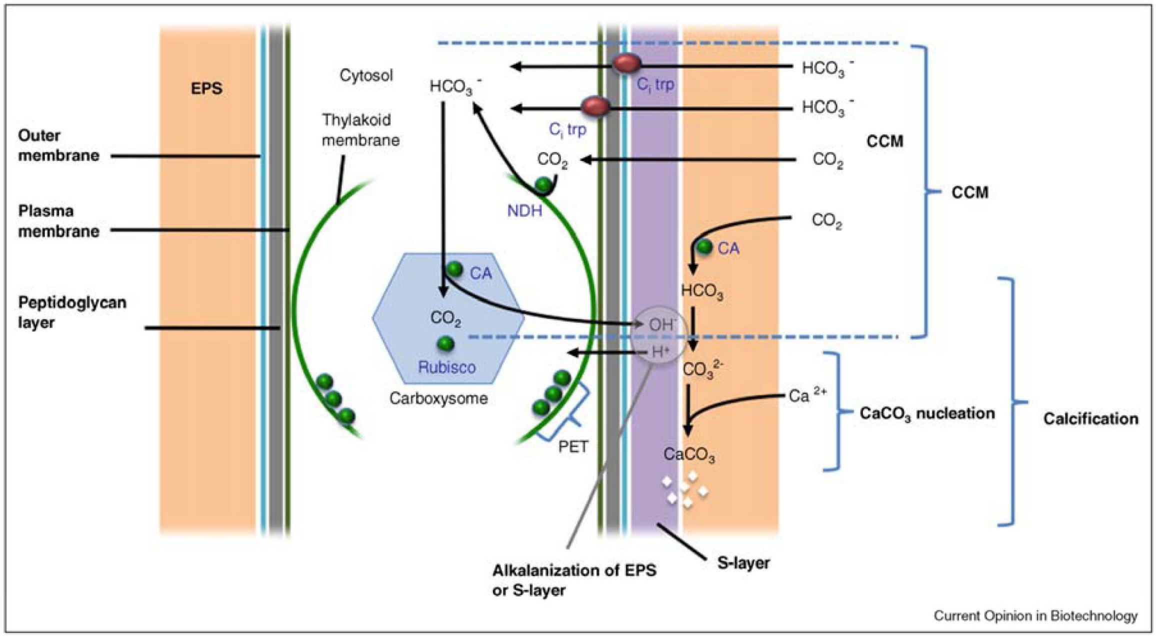

Fig. 1. Model of the carbon-concentrating mechanism (CCM) and calcification in a cyanobacterial cell. CO_2 enters the cells mainly via active transport of HCO_3^- and also through diffusion of CO_2, which is converted to HCO_3^- during the uptake. Cytosolic HCO_3^- is subsequently imported to the carboxysome. CA, carbonic anhydrase; C_i, inorganic carbon; EPS, exopolysaccharide sheath; NDH, NADPH dehydrogenase; and PET photosynthetic electron transport. Modified from Riding (2006) [47].

ecological change throughout Earth's history, and they continue to contribute to a large share of the total photosynthetic harnessing of solar energy and assimilation of CO_2 to organic compounds. For example half of global photosynthesis is carried out by phytoplankton, which mostly consist of cyanobacteria [14]. Indeed, 25% of global photosynthesis can be accounted for by the two marine cyanobacterial genera, *Synechococcus* and *Prochlorococcus* [15]. Our oxygenic atmosphere was originally generated by numerous cyanobacteria during the Archaean and Proterozoic Eras. Cyanobacteria generally thrive in high CO_2 levels and are considered as attractive systems for CO_2 capture from flue gas [16]. Many cyanobacteria are halophilic and, therefore, cyanobacteria for biofuel production or CCS can be cultured in marine waters, saline drainage water, or brine from petroleum refining industry or CO_2 injection sites, thereby sparing freshwater supplies. A large number of strains are thermophilic and thus tolerate high temperatures characteristic of flue gas. Also, being bacteria, cyanobacteria are amenable to homologous recombination, which allows rapid site-directed mutagenesis, gene insertions, replacements and deletions in a precise targeted and predictable manner.

Cyanobacteria and eukaryotic microalgae exhibit a CCM, a metabolic system that allows the cells to enrich the amount of CO_2 at the site of Rubisco (the first enzyme in the Calvin cycle that assimilates CO_2 into organic carbon compounds) up to 1000-fold over that in the surrounding medium [17-19]. The salient features of the CCM in cyanobacteria are shown in Fig. 1. Details differ between cyanobacteria, and the mechanisms are incompletely understood but the general arrangement consists of transport of HCO_3^-, the major uptake form of C_i in cyanobacteria, across the outer membrane and the plasma membrane, through HCO_3^-/Na^+ symports or ATP-driven uniports, as well as diffusion of CO_2, into the cytosol. Conversion of cytosolic CO_2 is carried out by NADPH dehydrogenase (NDH) complexes on the thylakoid and plasma membranes. HCO_3^- then enters the carboxysome, the protein-enclosed compartment that houses most of the Rubisco population, where it is converted to CO_2 in a reaction catalyzed by carbonic anhydrase (CA)

$CO_2 + H_2O \leftrightarrows H^+ + HCO_3^-$ (6)

The conversion of CO_2 to HCO_3^- via the NDH complexes relies on CA-like activities in associated proteins [18,20]. The active transport of HCO_3^- is dependent on extra ATP generated by cyclic electron transport around Photosystem I (PSI) in the photosynthetic electron transport chain (PET) [21-23]. The C_i transporters and the NDH complexes together constitute the combination of constitutive and inducible HCO_3^- uptake systems of the cyanobacterial CCM. When cells are exposed to CO_2/HCO_3^- limitation (<50 ppm CO_2), the inducible transport systems are activated, accompanied with increases in Rubisco activity and carboxysome content [20].

Interestingly, the explanation to why many cyanobacteria and eukaryotic microalgae have the ability to tolerate very high CO_2 concentrations, in some cases well above 50% CO_2 [21,24,25] might be found in the CCM. Inhibition of Rubisco through acidification under high CO_2 conditions is prevented by the CA reaction and by state II transition of PET (rearrangement of the phycobilisomes to favor light absorption by PSI) [21].

The idea of capitalizing on the high-CO_2 tolerance of cyanobacteria and microalgae for mitigation of CO_2 emissions in flue gas in connection with biofuel production was spawned already three decades ago [26,27] (and refs. therein). Since then, a large number of studies have been published where the potential for cyanobacterial and microalgal biofuels and beneficial CO_2 recycling is described and discussed [16,24,28-31]. Biomass production and CO_2 uptake in cyanobacteria and microalgae exposed to elevated CO_2 levels from flue gas or other streams have been followed for a variety of strains [16,29,31-36]. The overall conclusions from a large body of experiments are that: (1) cyanobacteria and microalgae can successfully assimilate significant amounts of CO_2 from sources such as flue gas; (2) many species are unaffected by the NO_x and SO_x present in flue gas; (3) thermophiles can be employed so as to minimize the cost of cooling the flue gas; (4) nutrients can be supplied via municipal wastewater to further reduce operation costs; and (5) both freshwater and marine species can be used.

Biomineralization by calcifying cyanobacteria

The occurrence and distribution of calcfying microorganisms are widespread [37-39]. A number of microbial strains capable of calcification have been reported, e.g. various cyanobacteria, eukaryotic microalgae, *Bacillus*, *Pseudomonas*, *Vibrio*, and sulfate-reducing bacteria. Although the phenomenon of microbial calcification has long been recognized, its physiological function is unknown. It might confer a selective advantage in providing a protective shield against high-light exposure [40], by offering a means for excretion of toxic levels of intracellular calcium [41], by enhancing nutrient uptake [40,42], or by serving as a buffer against pH rise in an alkaline environment [40], or increasing the uptake of CO_2 [43]. Since calcium is an important second messenger in cellular signaling, it is crucial that cells can control the flux of calcium in and out of cells, and calcification may be part of that regulatory process.

Calcification is particularly obvious in cyanobacterial species [40,44]. The geological and ecological significance of cyanobacterial calcification is immense [12,44-52]; spectacular examples of cyanobacterial calcification are stromatolites [53-55] and whitings, very fast, large-scale precipitations of fine-grained $CaCO_3$ together with organic compounds that can turn entire water bodies such as Lake Michigan and the Great Bahama Bank into a milky state [56-58]. Although our understanding of the molecular processes that trigger and control cyanobacterial calcification is hazy, and many of the mechanistic details of proposed models remain controversial, the general process is outlined in Fig. 1.

Cyanobacterial calcification is a non-obligate process that depends on photosynthetic activities, the CCM, extracellular surface properties, and environmental conditions [47,59]. Calcification might even be considered an integral part of the CCM. Calcification in cyanobacteria is an extracellular process and occurs on in the exopolysacccharide sheath (EPS) or proteinaceous surface layer (S-layer) that surrounds the cells [40,58,60-62]. Microenvironments of alkaline pH are generated at the EPS or S-layer owing to the CA activity in the carboxysome (reaction 6), which consumes H^+ (or produces OH^-) [63]. Other reactions that might contribute to local alkalinization of the EPS or S-layer are the PET (Fig. 1) and the plasma membrane-located Ca^{2+}/H^+ antiport, which transports Ca^{2+} out (and H^+ in) in an effort to maintain an optimal Ca^{2+} concentration in the cell [57]. The alkaline pH at the EPS or S-layer shifts the equilibria of the bicarbonate buffer system (reactions 4 and 5) to the right and promotes localized regions of increased CO_3^{2-} concentrations at the cell exterior. CA in the EPS [64] can further enhance local levels of HCO_3^- and CO_3^{2-} from incoming CO_2 or CO_2 that is leaked out from the cytosol [20]. In addition, both the EPS and S-layer contain Ca^{2+}-binding domains, e.g. glutamate and aspartate residues, which, together with the export of Ca^{2+} through the Ca^{2+}/H^+ translocator, raises the local Ca^{2+} concentration and serve as nucleation sites for $CaCO_3$ precipitation. Formed $CaCO_3$ can either precipitate as part of the EPS matrix or as calcified S-layers that shed from the cells, followed by subsequent synthesis of new S-layers. Cells that become completely embedded in $CaCO_3$ and die due to their inability to take up nutrients have also been observed [57].

An inspection of reaction 1 above shows that production of $CaCO_3$ results in the release of CO_2. Although less obvious, the same applies if carbonation proceeds from CO_3^{2-} (reaction 2) [43,65]. As a consequence, the partial pressure of CO_2 at the water surface rises. This calculation leads to the often puzzling and counterintuitive realization that $CaCO_3$ precipitation is associated with an increase in atmospheric CO_2. Simulations suggest that the *released CO_2:precipitated carbonate* ratio is close to 1 in freshwater but around 0.6 in marine waters, which are more buffered [43,65-67]. However, field and laboratory measurements revealed that biotic calcification exhibit *released CO_2:precipitated carbonate* ratios between 0.1 and 0.006 [43]. This agrees with careful experimental analyses of carbon flux during cyanobacterial calcification that showed a significant net CO_2 sequestration both in the field and laboratory [68]. The discrepancy between theoretic models and observed values most probably reflects the tight coupling between calcification and photosynthesis [43,66]. For example, the CO_2 released during calcification may be re-captured through photosynthesis [43]. Taking into account the combined effects of photosynthesis and calcification in seawater, Suzuki [66] presented a model showing that when the rate of photosynthetic biomass production (measured as *organic carbon production:calcification*) exceeds 0.6, the net effect is seawater absorption of atmospheric CO_2. On the contrary, for long-term carbon sequestration it is important that as much CO_2 as possible be routed to calcification rather than to organic compounds [57,69].

CCS using calcifying cyanobacteria

Through photosynthesis and calcification, cyanobacteria have the potential to capture CO_2 from flue gas and store it as precipitated $CaCO_3$. Calcium is abundant in many terrestrial, marine and lacustrine ecosystems. By using halophilic cyanobacteria, seawater or brines, e.g. agricultural drainage water, or saline water produced from petroleum production or geological CO_2 injections, can serve as potential calcium sources for the calcification process. Calcification can further be boosted by supplying calcium from gypsum [70] or silicate minerals, possibly in connection with biologically accelerated weathering [4].

However, successful implementation of calcifying cyanobacteria for point-source CCS are met with significant challenges that need to be addressed. For example, as seeing how alkalinization of the EPS or S-layer depends on HCO_3^- import (Fig. 1), the question arises as to whether calcification in cyanobacteria will occur also under high CO_2 conditions, e.g. when fed CO_2 from a flue gas stream. At high CO_2 levels, the CCM is not needed and cells will preferentially take up CO_2 rather than HCO_3^-. The conversion of CO_2 during transport to the cytosol (Fig. 1) produces H^+ (reaction 6) that needs to be neutralized, possibly via export to the medium [18]. This counterbalances the subsequent and opposite alkalinization reaction in the carboxysome. Also, rapid infusion of gaseous CO_2 into a cyanobacterial pond will probably lower the ambient pH, impeding alkalinization at the extracellular surface. Cyanobacteria still calcify under elevated CO_2 levels but photosynthesis seems to exert little or no influence on the process [13,57]. Furthermore, $CaCO_3$ precipitates were found to be more peripherally located on the extracellular surface and have a different morphology in cells predominantly taking up CO_2 instead of HCO_3^- [57,71]. Whether reactions such as PET and Ca^{2+} efflux suffice to generate extracellular alkaline microenvironments, to which extent CA activities in the EPS are involved, or if $CaCO_3$ precipitation during rapid CO_2 uptake becomes a passive process relying mainly of Ca^{2+} binding and nucleation at the EPS or S-layer, remains to be clarified.

It will be important to unravel the mechanisms of calcification and how they are regulated in cyanobacteria growing under flue gas conditions, and in the presence of pulverized gypsum or calcium silicate minerals. Strategies to promote HCO_3^- uptake would be to use strains where both the constitutive and inducible CO_2 uptake/conversion systems (Fig. 1) have been inactivated. Such mutants have been generated in *Synecococcus* PCC7942 and they exhibited HCO_3^- but no CO_2 uptake capacity [72]. Mutant cells grew at high CO_2 levels, but growth was not observed under CO_2-limiting conditions. Another option might be to have the flue gas pass through a CA system so as to convert incoming CO_2 to HCO_3^- prior to reaching the calcifying cyanobacteria. CA could either be overproduced and secreted as extracellular enzymes directly into the solution by cyanobacteria or other bacteria, or immobilized on solid supports.

Another issue relates to scale. A 500 MW coal-fired power plant emits between 3 and 4 Mt of CO_2 per year [73]. To be industrially relevant, ponds (or photobioreactors) with calcifying cyanobacteria have to produce large enough amounts of $CaCO_3$ to make an impact. Only a few attempts have been made at evaluating the rate of calcification in cyanobacteria. Extrapolating from whitings events in the Great Bahama Bank with an average of 70 km^2, and microcosm experiments with the marine *Synechococcus* 8806 (*S.* 8806), Lee et al. [7] estimated that calcification by *S.* 8806 could account for approximately 2.5 Mt $CaCO_3$ per year. This translates to sequestration of over half of the CO_2 produced from a 500 MW power plant [6,7]. Robust cyanobacterial strains or consortia need to be designed that exhibit maximized photosynthetic CO_2 uptake and that can fully utilize the plentiful calcium available in silicate minerals or gypsum. Calcification can be enhanced by increasing the number of carboxylate amino acids in the EPS that can be used as nucleation sites, and by increasing CA activities in the EPS. It is also crucial that strains be developed that have highly efficient light utilization and photoprotection properties. Cyanobacteria in general have low light requirements but when grown in ponds, cells below the surface will be light-limited while those at the top might experience excessive light intensities.

Furthermore, the information gained from studying calcification in cyanobacteria can be used for biomimetic approaches where artificial systems based on CA, CCM, EPS, or S-layers are designed for CO_2 capture and biomineralization. Crucial to these efforts is optimizing the long-term stability of the resulting carbonates [74]. For example, large calcite crystals containing an organic matrix similar to marine sediments are particularly stable and are highly desirable. Controlling the detailed morphology and composition of the organic (proteins, polysaccharide, etc.) and inorganic materials to result in highly stable carbonates is an important goal and may be achieved using biomimetic pathways to cyanobacterial mineralization. Ultimately such strategies could result in useful materials (i.e. bio-concrete).

Conclusions

Employment of cyanobacteria for point-source CCS of flue gas via calcification offers promising strategies for reducing anthropogenic CO_2 emissions. However, much research is urgently needed to further our understanding of the biochemical and physical processes in cyanobacteria that promote calcification, and that will allow us to select or design strains with optimized properties for specific applications and conditions using genetic engineering or directed evolution. For example, it is crucial that we determine the physiological functions of calcification in order to define conditions for maximal $CaCO_3$ production, and to be able to apply proper selection pressure for strain improvement. We also need to understand the different steps, that is nucleation, phase transition, crystallization, and aggregation in the biomineralization process, and the energy barriers for these stages so that we can identify bottlenecks in the overall process under different environmental conditions. We need to analyze the structural and functional characteristics of the EPSs and S-layers during calcification. We must investigate calcification at elevated CO_2 levels, such as in flue gas, and understand how photosynthetic light harvesting and photoprotection can be improved in cyanobacteria growing in open pond cultures or in photobioreactors under such conditions. We need to identify the genes involved in calcification and utilize available batteries of ‘omics technologies to obtain profiles for strains with different EPSs, S-layers, and capacities for calcification under various conditions.

Finally, it should not be expected that calcification by cyanobacteria and microalgae present an alternative to geological CCS. Rather biomineralization should most probably be viewed as a niche technology, preferably linked to small coal-fired power plants, natural gas systems, municipal solid waste combustion, and CO_2-emitting industries such as cement manufacture, and iron and steel production. If nation-wide distributions of such units were to be deployed in countries such as the U.S.A., China and India, the impact in mitigation of global greenhouse gas emissions could be enormous.

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