Selective Sequestration of Strontium in Desmid Green Algae by Biogenic Co-precipitation with Barite

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The generation of radioactive waste and/or environmental radioactive contamination is a common side effect of activities such as nuclear power generation, medical use of radioisotopes, nuclear weapons testing, and occasionally disasters such as Chernobyl. The subsequent decontamination of waste or the environment requires the nontrivial ability to selectively separate and remove harmful radioisotopes such as $^{90}$Sr, a product of nuclear fission with a half-life of approximately 30 years.[1] In the case of $^{90}$Sr, the chemical similarity of $\text{Ca}^{2+}$, $\text{Sr}^{2+}$, and $\text{Ba}^{2+}$ presents a challenge for even the most advanced ion-exchange materials.[2] While phytoremediation approaches utilizing the accumulation of environmental contaminants by green plants are becoming increasingly popular, the effectiveness of such approaches for $^{90}$Sr sequestration are drastically reduced in the presence of $\text{Ca}^{2+}$, due to the indiscriminate transport of $\text{Ca}^{2+}$, $\text{Sr}^{2+}$, and $\text{Ba}^{2+}$ exhibited by most organisms.[3]

Surprisingly, there are a small number of organisms that selectively sequester $\text{Sr}$ and/or $\text{Ba}$ in biominerals. For example, the marine radiolarian acantharea[4] builds an endoskeleton from celestite ($\text{SrSO}_4$), and the desmid[5] and stonewort[6] green algae deposit barite ($\text{BaSO}_4$) in vacuoles. Accumulation of $\text{Sr}$ and $\text{Ba}$ in the presence of up to five orders of magnitude excess $\text{Ca}$ emphasizes that to address the selectivity problem, there is much to be learned and possibly gained from the strategies these organisms have evolved. Here, we quantitatively demonstrate the incorporation of up to 45 mol% $\text{Sr}$ in barite crystals deposited by desmid green algae.

The unicellular desmid green algae are ubiquitous in fresh water habitats and robust in culture, and as such are particularly suitable as a model system for $\text{Sr}/\text{Ba}$ biomineralization and as a potential candidate for phytoremediation.[7] In the desmid Closterium moniliferum, $\text{BaSO}_4$ crystals are found in small terminal vacuoles at the tips of the crescent-shaped cells (Figure 1).[5] Scanning electron microscopy and energy dispersive spectroscopy (SEM/EDS) analysis of $\text{BaSO}_4$ crystals inashed cells reveals clusters of mixed rhombic and hexagonal crystals of 0.5–1 $\mu$m diameter with 0.1–0.5 $\mu$m thickness (Figure 1), which exhibit strong $\text{Ba}$ and $\text{S}$ signals and little $\text{Ca}$ (Supporting Information), consistent with previous descriptions.[5]

Wilcock and co-workers demonstrated, but did not quantify, $\text{Sr}$ incorporation into desmid crystals in a culture medium with a high ratio of $\text{Sr}^{2+}$ to $\text{Ba}^{2+}$.[5] This $\text{Sr}$ incorporation is a consequence of $\text{Sr}^{2+}$ substitution for $\text{Ba}^{2+}$ in the barite crystal lattice to form a ($\text{Ba},\text{Sr})\text{SO}_4$ solid solution.[8] In cultures grown in $\text{Ba}$-supplemented medium (0.1 $\mu$m) for several months, we observed multiple crystals in the vacuoles of almost all cells, while substitution of the same concentration of $\text{Sr}^{2+}$ for $\text{Ba}^{2+}$ in the medium resulted in virtually crystal-free cells. Thus, the presence of $\text{Ba}$ appears to be a prerequisite for crystal growth.

We quantitatively examined the elemental compositions of desmid crystals using synchrotron X-ray fluorescence (SXRF) microscopy. The high penetration depth, low detection limit, and small spot size of the X-ray microprobe[9] allow for imaging and compositional characterization of crystals within whole cells, with sensitivity to trace impurities. This direct imaging of individual crystals in situ is ideal for investigating the sequestration of $\text{Sr}$ and $\text{Ba}$ at the cellular level. In this way, one can avoid the artifacts associated with extracting and dissolving crystals from the extraordinarily mechanically and chemically robust cells for bulk elemental analysis.

SXRF elemental mapping was performed on whole plunge-frozen, freeze-dried $\text{C. moniliferum}$ cells. In elemental maps of cells cultured in $\text{Ba}$-supplemented medium, numerous $\text{BaSO}_4$ crystals are apparent as $\text{Ba}/\text{S}$ hotspots in the terminal vacuoles and throughout the cells (Figure 2). In cells cultured in...
medium supplemented with both Ba$^{2+}$ and Sr$^{2+}$ (at 0.1 $\mu$m each), Sr is co-localized with Ba and S in these hotspots, as expected for a (Ba,Sr)SO$_4$ solid solution crystal. Quantification of crystal compositions reveals Sr incorporation of 0.3 mol% ($X_{Sr} = 0.003$) relative to Ba. Notably, the culture medium in this case is undersaturated with respect to (Ba,Sr)SO$_4$. Formation of crystals thus requires that the cell increases the vacuolar sulfate concentration or dynamic effects of Sr and Ba transport to above their concentrations in the medium such that the activity product is greater than the solubility product of (Ba,Sr)SO$_4$.

The level of Sr incorporation into the desmid crystals at a given aqueous Ba mole fraction ($X_{Ba,aq}$) can be rationalized under the assumption that biological control over mineralization is limited. This has indeed been suggested based on the similarity of crystal habits observed in crystals grown biologically and in vitro. Thus, we consider the nucleation behavior in the vacuole in terms of equilibrium thermodynamics, which can be represented by a Lippman diagram (Figure 3). According to the Lippman diagram, the solubility product for (Ba,Sr)SO$_4$ decreases dramatically from pure SrSO$_4$ as Sr incorporation into these crystals is non-reversible, unlike, for example, uptake into the cytoplasm, binding to the cell wall, or extracellular precipitation. The observed low level of Sr incorporation ($X_{Sr} = 0.003$) at an $X_{Ba,aq}$ close to 0.5 is consistent with the prediction by the Lippman diagram.

Wilcock et al. reported that the morphology and composition of desmid crystals are influenced by the organism’s external ionic environment. If ion concentrations in the vacuole can be altered by changing the culture medium, we expect to induce precipitation of Sr-rich crystals ($X_{Sr} > 0.1$) when reducing $X_{Ba,aq}$ and increasing the activity product of the medium. To test this hypothesis, we exposed desmids to medium supplemented with 0.17 mM Sr$^{2+}$ and 0.35 mM Ba$^{2+}$ for 30–40 min. Following the exposure, cells were either harvested immediately or returned to regular medium and allowed to incubate for 35 min, 145 min, or 6.5 h before being fixed for analysis. We observed that crystals produced by these cells range in composition from 20 to 45 mol% Sr, with an average of 30 mol% Sr. Such crystals appear as Sr hotspots in SXRF images regardless of the post-exposure incubation time, and thus even after extensive washing (6.5 h) of cells with Sr-free medium. This demonstrates that Sr incorporation into these crystals is non-reversible, unlike, for example, uptake into the cytoplasm, binding to the cell wall, or extracellular precipitation.

The increase of Sr incorporation by more than two orders of magnitude under these conditions is consistent with the Lippman diagram and suggests that reducing the aqueous Ba mole fraction ($X_{Ba,aq} = 0.002$) and raising the activity product ($AP = 5.1 \times 10^{-8}$ m$^3$) of the medium effected similar changes in the vacuole. The shallow slope of the solidus line of the Lippman diagram for $X_{Sr} \approx 0.8$ may contribute to the large observed range ($X_{Sr} = 0.2–0.45$): small changes in the activity product in the vacuole, most likely influenced by slight variations in the vacuolar sulfate concentration or dynamic effects of Sr and Ba transport in individual cells, may lead to significant differences in the amount of Sr sequestered.
A comparison of Sr and Ca content in Sr-rich crystals clearly shows preferential incorporation of Sr (Figure 2). While crystals appear as hotspots in Sr maps, they are barely discernible against the background in Ca maps. This background is due to relatively high amounts of intracellular and cell wall-bound Ca that contributes to the overall signal in the columnar region of the cell sampled by SXRF. In a purely inorganic precipitation near equilibrium, the preferential incorporation of Sr over Ca in BaSO₄ is expected: there is a substantial difference in ionic radii, and CaSO₄·2H₂O forms a different crystal structure than the barite structure adopted by (Ba,Sr)SO₄. In fact, a miscibility gap is expected for a wide range of intermediate compositions of (Ba,Ca)SO₄, permitting incorporation of no more than 5 mol% Ca into BaSO₄.[14]

Desmids are thus attractive candidates for bioremediation of low-level radioactive effluents, as they appear to combine two familiar Sr sequestration techniques: phytoextraction and inorganic co-precipitation of Sr with barite. The integration of these two processes presents an optimized solution to some of the issues surrounding each technique individually (such as Ca-sensitivity and the need for microfiltration of fine precipitates, respectively).[3,15] Furthermore, traditional co-precipitation techniques require the elevation of ion concentrations in the entire receiving body of water in order to exceed the solubility product.[15] While we have used the elevation of Ba and Sr ion concentrations in the medium to drive up supersaturation in the vacuole, it is conceivable that with a greater understanding of ion transport and precipitation processes in desmids, this could be accomplished by more sophisticated means, such as by engineering the system to increase the sulfate concentration in the vacuole. Sr sequestration may also be enhanced by increasing the total amount of mineral produced simply by a small increase of the Ba concentration in the medium. Whether desmids have the necessary radiation tolerance for a phytoremediation approach is yet to be determined, although these organisms have proven to be resistant to harsh environments such as extreme temperature, acidic pH, low nutrient availability, and light limitation.[31]

To initially examine the feasibility of a desmid-based phytoremediation system, we estimate the total amount of co-precipitated Sr in cells that precipitated Sr-rich crystals in response to the 30–40 min exposure to 0.17 mM Sr²⁺ and 0.35 μM Ba²⁺ described above: this number ranges from 0.15 to 1.8 fmol per cell, averaging 0.85 fmol per cell. In a suspension culture of reasonable density (10⁷ cells L⁻¹) in which half of the cells contain crystals, this corresponds roughly to 4.3 nmol (or 0.38 μg) of sequestered Sr per liter of culture. By comparison, vetiver grass (Vetiveria zizanioides) plantlets have been reported to remove 50% of the ⁹⁰Sr from a solution spiked with 5× 10⁻⁹ kBq L⁻¹ after 1 h, which equates to 50 ng L⁻¹ of ⁹⁰Sr.[12] Notably, the Sr-rich crystals in the desmids analyzed here were formed after a 30–40 min exposure, and more sequestration is likely for longer exposure times; cells cultured long-term in Ba-supplemented medium have been known to contain 20 or more crystals per cell. In addition, the analysis here does not take into account soluble Sr⁺ within the cell or adsorbed to the cell wall. A thorough exploration of the effect of various culture conditions on total Sr sequestration in bulk cultures is therefore needed, and may lead to the classification of C. moniliferum (and possibly other desmids) as Sr hyperaccumulators.

In summary, we quantitatively demonstrate the sequestration of Sr in desmids; permanent removal of Sr from solution could be ensured simply by harvesting cells after crystal precipitation and isolating crystals by filtration and/or ashing (as in Figure 1c). In addition, we show that it is possible to tailor biogenic crystal compositions by altering medium compositions based on the nucleation characteristics of the (Ba,Sr)SO₄ solid solution. A detailed investigation into (1) the capabilities of desmids for bulk removal of Sr and Ba from contaminated water and (2) the physiological and molecular mechanisms at play in desmids and other Sr/Ba-mineralizing organisms is clearly warranted, and is likely to lead to innovations in biotechnological and bio-inspired approaches to the safe removal of toxic metals from the environment.

**Experimental Section**

Closterium moniliferum cultures were obtained from The Culture Collection of Algae at the University of Texas at Austin (UTEX). Cultures were maintained in Bold’s Basal Medium[16] with 3-fold nitrogen and vitamins (3NBBM+V) at 20–25 °C in a 12 h light/dark cycle under 20 Wm⁻² of daylight-spectrum fluorescent light. Algae were transferred to fresh medium every 1–3 months. All cultures, whether in deprived or Ba/Sr-supplemented medium, were slowly dividing (i.e., doubling times were at least several days.) Cells that were virtually free of crystals were obtained by growth in Ba- and Sr-deprived medium for several weeks to several months[5,17] and were used as the starting point for Ba/Sr exposures (unless otherwise noted). The culture medium composition and experimental Ba/Sr concentrations are given in the Supporting Information.

For SEM imaging, cells were allowed to settle on a silicon wafer and ashed in a box furnace at 450 °C for 1.5–2 h. Samples were analyzed uncoated by using an S-4800-II field emission scanning electron microscope (Hitachi Ltd, Tokyo, Japan). For X-ray imaging, cells were allowed to settle on silicon nitride windows (area 1.5 x 1.5 mm², thickness 500 nm, Silson, Bilsworth, UK) for 15 min and processed by cryofixation. Windows were blotted once (0.5 s, 0 bl ot force) with filter paper to remove excess water and plunge-frozen into liquid ethane using a Vitrobot Mark IV (FEI Company, Hillsboro, OR). Samples were then freeze-dried in an EMS 775 turbo freeze-dryer (Electron Microscopy Sciences, Hatfield, PA) during slow warming from −140 °C to 20 °C over 12 h. Freeze-dried Closterium samples were analyzed using the X-ray microprobe at beamline 2-ID-E of the Advanced Photon Source (Argonne, IL). A crystal monochromator was used to select the energy of the beam, and a Fresnel zone plate (320 μm diameter, 100 nm outermost zone width, X-radia, Concord, CA) focused the beam onto a submicron spot size (depending on the incident energy). The sample was then raster-scanned through the beam, and a full fluorescence spectrum was acquired at each point with a 1 s dwell time using an energy dispersive fluorescence detector (Ultra-LE Ge detector, Canberra, Meriden, CT or Vortex EM, SSL Nanotechnology, Northridge, CA). For imaging of cells from the Ba-supplemented condition, an incident energy of 10 keV was used, with a step size of 0.5 μm. For imaging Sr-supplemented cells, an incident energy of 17.5 keV was used, with a step size of 1.25 μm, in order to excite the Sr K-edge.[18] Elemental maps were generated and peak fitting and quantification were performed using MAPS software.[19]
The fluorescence signal was converted to area concentration by fitting sample spectra against the spectra collected from thin-film standards (NBS-1832 and NBS-1833, National Bureau of Standards, Gaithersburg, MD). Crystal compositions were determined by reading quantified area concentrations for each S/Ba/Sr hotspot. Because the crystal dimensions were comparable to the step size of the SXRF scan, each crystal was defined by one pixel. Colocalization analysis was performed using the Colocalization plugin in ImageJ,[20] using a ratio of 50% and thresholds of 50 for both channels.

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