Combined *in vivo* and *in vitro* studies of $BaSO_4$ formation in green alga *Spirogyra*

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The formation mechanism of biogenic barites in fresh- and seawater has been under constant debate. As these barite particulates are found in undersaturated conditions [1], the most accepted hypothesis has been the creation of barite supersaturated microenvironments from the degradation of sinking organic matter [2]. Alternatively, the active barite biomineralization within phytoplankton and planktonic protists could be the biogenic origin of these particulates [3]. We investigated barite biomineralization in a freshwater green alga, *Spirogyra*, which is well known for its helical chloroplast. The presence of barite microcrystals in *Spirogyra* is often disregarded, while for its close relatives, *Closterium* and *Micrasterias*, it has been well documented [4,5]. We characterized the size, morphology, and location of these crystals within *Spirogyra* sp. cells by combining *in vitro* and *in vivo* techniques to propose their formation mechanism.

First, scanning electron microscope (SEM) and energy-dispersive X-ray spectroscopy (EDXS) were performed on dried filaments. The smaller crystals showed a fast-growing face, (011), which is typically not observed for *in vitro* barites. This suggests an influence of organic molecules on the growth kinetics, before reaching the final rhombic morphology with (210) and (001) faces. Then, the critical point drying method was applied to preserve the structure of *Spirogyra* cells for SEM imaging. Microcrystals were found against the cytoplasmic membrane, close to chloroplasts and fibrillary network. Finally, optical microscopy and Raman tweezer microspectroscopy were used to identify their location *in vivo*. Many moving micro-particles were found optically at the top, sides and bottom of the cell, implying that they are located in cytoplasm. These particles were optically trapped and their measured Raman spectra matched sulfate peaks, assigned as barite from separate EDXS analyses. These barite microcrystals follow the cytoplasmic streaming, further confirming their location in cytoplasm [6].

The combined *in vitro* and *in vivo* studies allowed us to propose that barite formation in *Spirogyra* occurs in the cytoplasm. Barium could be supplied by a non-selective transport of divalent cations as observed in *Closterium* [4], while sulphate is transported to chloroplasts for amino acid biosynthesis. This study plays a key step toward understanding if these crystals have a biological function in *Spirogyra*.

References

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