TEM-specimen preparation of cell/mineral interfaces by Focused Ion Beam milling

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ABSTRACT

Picocyanobacteria were found to play an important role in calcite precipitation in oligotrophic lakes. In this study, investigations on the interface between cyanobacteria and attached biogenic calcite crystals have been performed to gain further insights into the mechanisms of nucleation of these precipitates. Ultramicrotomy, the conventional preparation technique of thin sections for Transmission Electron Microscopy (TEM) investigations, often fails when working on heterogeneous samples containing soft organic material and hard minerals. Thus, in this study the thin sections were prepared using Focused Ion Beam (FIB) milling. This approach is usually applied in material sciences but until recently was not very common in environmental research. Different analytical TEM methods like Electron Spectroscopic Imaging (ESI) and Electron Energy Loss Spectrometry (EELS) were used to test the suitability of FIB-milling for the preparation of organic/inorganic interface specimens. With this approach we were able to analyze both organic and the inorganic phases of the same sample. Elemental maps of the samples were also calculated. By analyzing the structure of the C K-absorption edge, the different bonding forms of the organic carbon cell and the inorganic carbon of the crystal could be clearly distinguished.

INTRODUCTION

Lacustrine calcite precipitation with sedimentation rates of several g/m²d (calculated from Bloesch 1974) can result in large carbonate deposits. Varied lake sediments high in calcite content are now intensively studied as high-resolution continental archives for environmental change (Lotter et al. 1997). Eukaryotic and prokaryotic picoplankton species have been found to play an important role in the overall process of calcite precipitation, particularly in oligotrophic hardwater lakes.

Studies of biogenic calcite precipitation have been done from various perspectives. Several authors have analyzed environmental conditions leading to biogenic calcite precipitation (Canaveras et al. 2001; Merz-Preiss 2000; Castanier et al. 1999; Saiz-Jimenez 1999; Merz et al. 1995; Thompson and Ferris 1990; Thompson et al. 1997). Dittrich et al. (2004) combined field observations with laboratory experiments using several eukaryotic and prokaryotic plankton strains.

Besides these large-scale studies several microscopy studies have been performed. TEM investigations focused primarily on the paracrystalline proteinaceous surface layers (called S-layers) of cyanobacterial cells as a template for nucleation (Schultze-Lam and Beveridge 1994a, b; for review see Smarda et al. 2002). Schultze-Lam et al. (1992) suggest that as a first step hydrated Ca²⁺-ions are bound to the regularly spaced and negatively charged proteins of the S-layer. These suggestions were based on structural investigations of S-layers and “bulk”-analysis of their chemical composition.

However, in our study we developed a sample preparation method for analytical TEM studies of thin sections of the interface between picoplankton cell walls and the crystals attached to these cells. Investigations of the C K-absorption edge by TEM-EELS provide information about the bonding of carbon at this interface and could therefore possibly prove the involvement of negatively charged proteins in the bonding of Ca²⁺ ions to the cell surface.

Conventionally thin sections of environmental samples for TEM investigations are cut using ultramicrotomy (e.g., Schultze-Lam et al. 1992). The heterogeneity of samples containing both soft organic material and hard minerals complicates the use of this conventional approach because minerals are barely cut by the diamond blade without applying stress to the interface between soft and hard matter. Another disadvantage of ultramicrotomy is the randomness of positioning of the slice plane. To overcome these limitations on investigations of heterogeneous cell-mineral interfaces, thin sectioning by Focused Ion Beam (FIB) milling has been successfully applied to environmental particles (Benzerara et al. 2005; Mavrocordatos et al. 2003; Heaney et al. 2001). These sections were prepared for analytical TEM studies.

Nevertheless, this technique has to be adapted to and optimized for heterogeneous samples containing both soft organic matter such as cyanobacterial cells and biogenic mineral phases. Mulders (2003) showed that it was possible to mill fragile organic matter. Using a cryo stage he was able to visualize the inner structure of cross-sectioned bacteria without any chemical fixation. However, from Mulders (2003) it is not clear if the physico-chemical structure of the milled side wall is affected by the FIB or preserved.