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Impact of biomineralization on the preservation of microorganisms during fossilization: An experimental perspective

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ABSTRACT

The biogenicity of fossil microbial biomorphs is often debated because their morphologies are poorly informative and the chemical, structural and isotopic signatures of putative biogenic organic molecules have been altered during their incorporation into the sediments and the geological history of the host rock. Here, we investigated the effect of encrustation by biominerals on the morphological and chemical degradation of *Escherichia coli* cells during experimental thermal treatments. Non-calcified *E. coli* cells and *E. coli* cells encrusted by calcium phosphates were exposed to heating under an Argon atmosphere at two different temperatures (300 °C, 600 °C) for 20 h. Two additional experiments were performed on non-calcified *E. coli* cells at 300 °C for 2 h and 100 h to discuss the influence of experiment duration. Organic residues of all experiments were characterized at a multiple length scale using a combination of scanning electron microscopy, transmission electron microscopy, Raman microspectroscopy, electron paramagnetic resonance (EPR) spectroscopy, Fourier transform infrared (FT-IR) spectroscopy and X-ray absorption near edge structure spectroscopy (XANES) at the carbon K edge. In the absence of encrusting biominerals, the morphological structure of the organic residues of *E. coli* cells was completely lost after heating at 300 or 600 °C, even after short (2 h long) heating experiments. The content of aromatic functional groups of the organic residues of non-calcified *E. coli* cells increased during heat-treatment at 600 °C for 20 h while the amide functional groups were lost, as indicated by FT-IR spectroscopy. Consistently, the EPR spectrum of these organic residues indicated important transformation. As a comparison, this spectrum appeared similar to EPR spectra of ancient organic carbons such as carbons from the Apex chert (ca. 3460 Myr), indicating a similar concentration of aromatic moieties. In contrast, calcified *E. coli* exposed to the same conditions showed only limited morphological alteration as observed by electron microscopy as well as lower chemical transformation as detected by FT-IR and EPR spectroscopies. Despite the difficulties to relate experimental conditions directly to geological conditions, these experiments evidence the influence of cell encrustation by minerals on their chemical and morphological preservation potential during fossilization processes.

1. Introduction

A wide diversity of microorganisms can form a variety of crystalline or amorphous biominerals via biomineralization within their cells, in their cell walls, at the cell surface, on extracellular structures or away from the cells (e.g., Lowenstam, 1981; Weiner and Dove, 2003; Konhauser and Riding, 2012). These biomineralization processes are relatively fast and can occur in a few hours or days (e.g., Miot et al., 2009; Chan et al., 2011), resulting in the formation of diverse organo-mineral complexes including biominerals and biomineral-encrusted cells/biostructures (J.H. Li et al., 2013). Encrustation of microbial cells by biominerals can be seen as a first stage of fossilization (e.g., Oehler and Schopf, 1971; Westall et al., 1995; Toporski et al., 2002; Benning et al., 2004; Lalonde et al., 2005; Miot et al., 2009; Orange et al., 2009; Li et al., 2010; Chan et al., 2011).

It has often been hypothesized that biomineral-encrusted cells/biostructures may be more resistant to diagenesis and/or metamorphism than pure organic molecules and structures, and

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thus better preserved over geological timescales (e.g., Ferris et al., 1988; also see J.H. Li et al., 2013 for a review). Noteworthy, early diagenetic phosphatization, i.e., phosphate permineralization occurring prior to the degradational collapse of cells and cellular tissues, may favor their structural preservation (e.g., Butterfield et al., 2007). Micro-organisms fossilized in phosphorites have been reported for ages. The most emblematic examples are undoubtedly the ~570-million-year-old fossils of the Ediacaran Doushantuo phosphorite formation in China (e.g., Xiao et al., 1998; Xiao and Knoll, 2000; Huldtgren et al., 2011). A long time before this discovery, Cayeux (1936) reported fossils of bacteria in phosphorites and suggested that they might be present in phosphorites throughout the geological record. Since then, there has been a high interest in microfossils in phosphorites, especially in the last few years (e.g., Zanin and Zamirailova, 2011; Cosmidis et al., 2013a, 2013b; She et al., 2013; Bailey et al., 2013).

Assessing the biogenicity of microbial-like structures in ancient rocks remains challenging since the original biological information they contain can be significantly altered during fossilization processes (e.g., Gourier et al., 2004; Vandenbroucke and Largeau, 2007; Papineau et al., 2010), and these biostructures may thus be mistaken with abiotic structures (Brasier et al., 2002; García-Ruiz et al., 2003; Van Zuilen et al., 2007; Cosmidis et al., 2013a). In addition, determining unambiguously the syngenicity of putative microfossils within a rock requires a precise assessment of the thermal history that they have experienced (Brasier and Wacey, 2012).

Exposing modern microorganisms in the laboratory to well-constrained temperature conditions may help improving our knowledge on the transformations of such fossils induced by burial and maturation processes (e.g., J.H. Li et al., 2013). Such experiments have been increasingly used to study the thermal maturation of biomolecules or biominerals under various pressure and temperature conditions (e.g., Stankiewicz et al., 2000; Gupta et al., 2006; Skrzypczak-Bonduelle et al., 2008; Watson et al., 2012; Bourbin et al., 2013a; Y.L. Li et al., 2013). For instance, Skrzypczak-Bonduelle et al. (2008) reported the continuous evolution of electron paramagnetic resonance (EPR) line shapes gradually from a Gaussian–Lorentzian to a stretched-Lorentzian profile for organic matter experiencing step-heating from room temperature up to 800 °C, similarly to what is reported for field samples. Similar results were recently obtained by Bourbin et al. (2013a) on bacteria heat-treated in the laboratory, thereby confirming that EPR spectroscopy provides a powerful tool to study the geochemical maturity of organic matter.

However, comparing extant microbes or biominerals with putative ancient microfossils or fossil biominerals is still a challenge as the impact of mineral encrustation on the preservation of microbial fossils has not yet been tested in detail (e.g., J.H. Li et al., 2013). Although a number of studies have been dedicated to the impact of silicification on the preservation of organic biostructures for instance, most of these studies have been focusing on the morphological preservation of organic structures (Oehler and Schopf, 1971; Ferris et al., 1988; Westall et al., 1995; Toporski et al., 2002; Lalonde et al., 2005; Orange et al., 2009). Recent analytical advances now allow for characterizing chemically and mineralogically these objects down to the nm-scale, sometimes in 3D (e.g., Miot et al., 2014; Wacey et al., 2014) and thus allow exploring the evolution of organic biosignatures during artificial maturation.

Here, we report results of thermal degradation experiments performed on *Escherichia coli* cells with or without Ca-phosphate encrusting their cell walls at 300 and 600 °C under argon atmosphere for 20 h. Two additional experiments were performed on non-calcified *E. coli* cells at 300 °C for 2 h and 100 h to discuss the influence of experiment duration. Organic residues of all experiments were characterized using highly complementary techniques, namely scanning electron microscopy (SEM), transmission electron microscopy (TEM), Raman microspectroscopy, EPR spectroscopy, Fourier transform infrared spectroscopy (FT-IR) and scanning transmission X-ray microscopy (STXM) coupled with X-ray absorption near edge structure (XANES) spectroscopy. While Raman spectroscopy is sensitive to the organization of the aromatic skeleton in carbonaceous material (e.g., Beyssac et al., 2003; Lahfid et al., 2010; Beyssac and Lazzeri, 2012), EPR spectroscopy provides information on paramagnetic defects in carbonaceous materials with high sensitivity (e.g., Mrozowski, 1988; Bourbin et al., 2013a), C-XANES spectroscopy provides information on organic carbon speciation (e.g., Bluhm et al., 2006; Solomon et al., 2009), and FT-IR spectroscopy allows identifying the different chemical bonds within organic molecules (e.g., Yule et al., 2000).

2. Materials and methods

2.1. Sample preparation

Ca-phosphate encrusted (i.e., calcified) and non-encrusted (i.e., non-calcified) *E. coli* cell samples used in the present study were derived from the same *E. coli* Apm1 strain. Strain Apm1 was obtained at IMPMC by transformation of *E. coli* BL 21 cells by the pET gene expression system (Studier and Moffatt, 1986). This plasmid contained (i) a *phoa* gene coding for an alkaline phosphatase linked to a promoter activated by isopropyl β-D-thiogalactoside (IPTG), and (ii) an ampicillin resistance marker. The addition of IPTG to the culture medium induced the transcription of the *phoa* gene, resulting in the over-expression of alkaline phosphatase enzymes (PHO A). Encrustation of the cells by Ca-phosphates was achieved by culturing cells over-expressing PHO A in a calcification medium.

To obtain cells encrusted by Ca-phosphates, cells from one single colony grown on LB agar medium (Sigma-Aldrich Co.), were inoculated within 5 ml of autoclaved liquid LB medium (Sigma-Aldrich Co.) and grown overnight at 37 °C and 180 rpm. This 5-ml culture was transferred into 500 ml of liquid LB for bacterial amplification culture. This culture was also carried out at 37 °C and 180 rpm; the optical density at 600 nm (OD_{600}) was monitored during the growth. Once the OD_{600} reached a value between 0.6 and 0.8 after ~4–6 h incubation (corresponding to the exponential growth phase of *E. coli* cells), 1 ml of IPTG (0.4 mM) was added to induce the overexpression of the *pho a* gene, leading to the production by the cells of high amounts of PHO A. After 4 h of incubation under these conditions, cells were harvested by centrifugation at 6500 rpm for 15 min and rinsed three times with distilled water. Cells were then transferred to 2 l of a calcification medium and incubated at 37 °C with shaking at 120 rpm. The calcification medium, specifically designed to favor encrustation by Ca-phosphates, was prepared by dissolving calcium glycerophosphate (glycerol phosphate calcium salt, Sigma-Aldrich Co.) in a HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) pH-buffer (20 mM). HCl (1 M) was used to adjust the pH to 7.5 and the medium was sterilized by filtration through a 0.22-µm filter. After a week, the incubated cells, completely encrusted by Ca-phosphates were centrifuged at 8000 rpm for 15 min and rinsed three times in distilled water.

To obtain the non-encrusted *E. coli* cells, 2 ml of precultured Apm1 suspension were transferred into 1 l of liquid LB medium and allowed to grow overnight, and then harvested by centrifugation and rinsed with distilled water using the same protocol as for the calcified *E. coli*.

All media contained a final concentration of ampicillin of 50 µg ml^{-1} to prevent growth of potential contaminants.