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Microscopy evidence of bacterial microfossils in phosphorite crusts of the Peruvian shelf: Implications for phosphogenesis mechanisms

Julie Cosmidis [a,b], Karim Benzerara [a,*], Nicolas Menguy [a], Esther Arning [c]

[a] Institut de Minéralogie et de Physique des Milieux Condensés, UMR CNRS 7590-UPMC-IRD, 4 place Jussieu, 75252 Paris cedex 05, France
[b] Institut de Physique du Globe de Paris, UMR CNRS 7154 — Paris 7, 1 rue Jussieu, 75238 Paris cedex 05, France
[c] Helmholtz Centre Potsdam — GFZ German Research Centre for Geosciences, Telegrafenberg, 14473 Potsdam, Germany

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ABSTRACT

Phosphorites are sedimentary formations enriched in Ca-phosphate minerals. The precipitation of these minerals is thought to be partly mediated by the activity of microorganisms. The vast majority of studies on phosphorites have focused on a petrological and geochemical characterization of these rocks. However, detailed descriptions are needed at the sub-micrometer scale at which crucial information can be retrieved about traces of past or modern microbial activities. Here, scanning electron microscopy (SEM) analyses of a recent phosphorite crust from the upwelling-style phosphogenesis area off Peru revealed that it contained a great number of rod-like and coccus-like shaped micrometer-sized (~1.1 μm and 0.5 μm, respectively) objects, referred to as biomorphs. Some of these biomorphs were filled with carbonate fluoroapatite (CFA, a calcium-phosphate phase common in phosphorites); some were empty; some were surrounded by one or two layers of pyrite. Transmission electron microscopy (TEM) and energy dispersive X-ray spectrometry (EDXS) analyses were performed on focused ion beam (FIB) milled ultrathin foils to characterize the texture of CFA and pyrite in these biomorphs at the few nanometer scale. Non-pyritized phosphatic biomorphs were surrounded by a thin (5–15 nm thick) rim appearing as a void on TEM images. Bundles of CFA crystals sharing the same crystallographic orientations (aligned along their *c*-axis) were found in the interior of some biomorphs. Pyrite formed a thick (~35–115 nm) layer with closely packed crystals surrounding the pyritized biomorphs, whereas pyrite crystals at distance from the biomorphs were smaller and distributed more sparsely. Scanning transmission X-ray microscopy (STXM) analyses performed at the C K-edge provided maps of organic and inorganic carbon in the samples. Inorganic C, mainly present as carbonate groups in the CFA lattice, was homogeneously distributed, whereas organic C was concentrated in the rims of the phosphatic biomorphs. Finally, STXM analyses at the Fe $L_{2,3}$-edges together with TEM-EDXS analyses, revealed that some pyritized biomorphs experienced partial oxidation. The mineralogical features of these phosphatic biomorphs are very similar to those formed by bacteria having precipitated phosphate minerals intra- and extracellularly in laboratory experiments. Similarly, pyritized biomorphs resemble bacteria encrusted by pyrite. We therefore interpret phosphatic and pyritized biomorphs present in the Peruvian phosphorite crust as microorganisms fossilized near the boundary of zones of sulfate reduction. The implications of these observations are then discussed in the light of the different possible and non-exclusive microbially-driven phosphogenesis mechanisms that have been proposed in the past: (i) Organic matter mineralization, in particular mediated by iron reducing bacteria and/or sulfate-reducing bacteria (SRB), (ii) reduction of iron-(oxyhydr)oxides by iron-reducing bacteria and/or SRB, and (iii) polyphosphate metabolism in sulfide-oxidizing bacteria, possibly associated with SRB.

1. Introduction

Phosphorites are phosphorus-rich sedimentary rocks, representing a major sink in the global biogeochemical cycle of P (Follmi, 1996; Paytan and McLaughlin, 2007). Phosphorites are known to have formed at different periods in the history of the Earth, from the Paleoproterozoic to present days (Filippelli, 2008; Papineau, 2010; Filippelli, 2011). Most of the large modern marine phosphorites form in oceanic upwelling regions characterized by an important organic sedimentation, as observed off the western coasts of Peru and Chile, Mexico and Namibia (Baturin and Bezrukov, 1979; Follmi, 1996, and references therein). Phosphorite formation is driven by the precipitation of carbonate fluoroapatite (CFA) in the sediment pore solution during early diagenesis close to the sediment–water interface (Froelich et al., 1988; Glenn, 1990; Reimers et al., 1996). It is assumed that bacteria play a crucial role in phosphogenesis by increasing interstitial phosphate

* Corresponding author at: Institut de Minéralogie et de Physique des Milieux Condensés, 4 place Jussieu, 75252 Paris cedex 05, France. Tel.: +33 1 44 27 75 42.
E-mail address: karim.benzerara@impmc.upmc.fr (K. Benzerara).

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concentrations through degradation of organic matter (Burnett, 1977; O'Brien et al., 1981; Krajewski et al., 1994; Follmi, 1996). Sulfate-reducing bacteria (SRB), which are responsible for the greater part of organic matter remineralization in most marine sediments, may be particularly important in this process (Glenn, 1990; Arning et al., 2009a; Berndmeyer et al., 2012). Other bacterial metabolisms, such as dissimilatory Fe reduction may however play a significant role as well (*e.g.*, Thamdrup, 2000). Increased phosphate concentrations in porewater, conducting to CFA precipitation, can also be achieved by iron redox pumping, a mechanism in which phosphate is released following the bacterial or abiotic reductive dissolution of iron-(oxyhydr)oxides in the sediments (Froelich et al., 1988; Schuffert et al., 1998; Scopelliti et al., 2010). Another model proposes that the activity of large polyphosphate-accumulating sulfide-oxidizing bacteria drives phosphogenesis in upwelling region off Namibia (Nathan et al., 1993; Schulz and Schulz, 2005; Goldhammer et al., 2010).

The involvement of bacteria in past phosphogenesis is supported by the frequent occurrence of putative fossil microorganisms in phosphorites (Soudry, 2000; Zanin and Zamirailova, 2011; and references therein). However, the biogenic (*vs.* abiotic) nature of these micrometer-sized biomorphic objects is often debated (Baturin and Titov, 2006). Sub-micrometer scale mineralogical and textural characterization of these objects sometimes provides additional evidence in support of a bacterial interpretation (Cosmidis et al., 2013).

Arning et al. (2009b) studied the geochemistry and petrology of recent authigenic phosphorite crusts from the upwelling area off Peru (dating from Middle Miocene to Pliocene) in order to constrain the environmental conditions of their formation. They proposed that CFA composing these phosphorites precipitated under suboxic conditions close to the sediment–water interface, with development of episodic anoxic conditions driving the formation of accessory sulfide minerals (pyrite FeS_2 and sphalerite ZnS) by bacterial sulfate reduction. Sulfur stable isotope compositions and molecular biomarkers further demonstrated the abundance of SRB in these rocks and suggested concurrent bacterial sulfide oxidation (Arning et al., 2009a). Large sulfide-oxidizing bacteria frequently occur on the seafloor of upwelling areas off Namibia, Peru and Chile and are found in close association with SRB in the sediments of these regions (Arning et al., 2008, and references therein). A phosphogenesis mechanism involving SRB and sulfide-oxidizing bacteria has therefore been proposed for the Peruvian shelf phosphorite formation area.

The aim of the present study is to investigate possible textural traces of these microbial processes using a fine-scale microscopy characterization of these phosphorite crusts. This may serve as a basis for future studies searching for traces of microorganisms in older phosphorites (*e.g.*, Sánchez-Navas and Martín-Algarra, 2001; Trela, 2008; Rozanov and Astafieva, 2009). Here, we applied high spatial and spectral resolution spectromicroscopy techniques such as transmission electron microscopy (TEM), scanning transmission electron microscopy (STEM) coupled with energy dispersive X-ray spectrometry (EDXS), focused ion beam (FIB) milling and scanning transmission X-ray microscopy (STXM) on phosphorite crusts collected on the Peru margin in order to (i) identify textural traces of life preserved in recent phosphorites and which may serve as references for the study of older samples and (ii) gain further understanding of phosphorite formation models.

2. Material and methods

2.1. Material

The sample is a fragment of phosphorite crusts collected within the oxygen minimum zone of the Peru margin (10°01.89′S; 79°04.33′W), at a 179 m water depth (area A in Fig. 1 of Arning et al., 2009b). The crusts were sampled during the SONNE cruise SO-147 using a TV-Grab and a box corer. A detailed description of the petrography and geochemistry of similar samples collected in the same area can be found in Arning et al. (2009b). These phosphorite crusts formed during a period of time lasting from Middle Miocene to Pleistocene.

2.2. Scanning electron microscopy

A section of a phosphorite crust was polished and coated with carbon. Scanning electron microscopy (SEM) analyses were performed using a Zeiss Ultra 55 equipped with a field emission gun. Backscattered electron (BSE) images were acquired at 10 kV with a working distance of 7.5 mm using an AsB detector. Energy-dispersive X-ray spectrometry (EDXS) analyses were performed using an EDXS QUANTAX microanalyzer (Brucker). X-ray maps were acquired as hypermaps using drift correction in the Esprit software.

2.3. Focused ion beam milling

Five electron-transparent foils were prepared at CP2M (Centre Pluridisciplinaire de microscopie électronique et de microanalyse, Université d'Aix-Marseille, Marseille, France) by focused ion beam (FIB) milling using a Phillips FIB 200 instrument operating at 30 keV and 5 nA following the FIB lift-out method (Heaney et al., 2001). Foils were further thinned to ~100 nm with a glancing angle Ga ion beam at lower beam current (approximately 50 pA) to remove the layer damaged by Ga ions. Before milling, a platinum strap was deposited at the surface of the sample.

2.4. Scanning transmission X-ray microscopy

The FIB foils were analyzed by scanning transmission X-ray microscopy (STXM) at the carbon K-edge (C K-edge) and at the iron $L_{2,3}$-edges (Fe $L_{2,3}$-edges). Analyses were performed on beamline 11.0.2.2. at the Advanced Light Source (Lawrence Berkeley National Laboratory, Berkeley, USA), using a 25 nm zone plate. Energy calibration was achieved using the well-resolved 3p Rydberg peak of gaseous CO_2 at 294.96 eV. Data included images and image stacks, from which XANES spectra and maps were retrieved. The aXis2000 software (Hitchcock, 2012) was used for data processing. Many previous studies have shown what information can be retrieved from C K-edge XANES coupled with STXM for the characterization of organic matter and the limits of this approach (*e.g.*, Lehmann et al., 2005; Takahama et al., 2007; Bernard et al., 2010; Remusat et al., 2012; Solomon et al., 2012).

Images of the samples were taken at 702 eV (below the Fe $L_{2,3}$-edges), 708 eV (maximal absorption energy of Fe(II)) and 709.9 eV (maximal absorption energy of Fe(II)). Fe is mainly present as Fe(II) in areas presenting higher absorption at 708 eV than at 709.9 eV (Bourdelle et al., 2013). In contrast, Fe contains mainly Fe(III) in areas presenting higher absorption at 709.9 eV than at 708 eV. This was used to assess the potential heterogeneities of the redox state of Fe in the samples. Pre-edge-corrected images converted in optical density (OD) at 708 eV and 709.9 eV were obtained by subtracting the OD-converted image obtained at 702 eV from the OD-converted image obtained at 708 eV and 709.9 eV respectively. Pre-edge-corrected OD images at 708 eV were subtracted from pre-edge-corrected OD images at 709.9 eV, and *vice-versa*. Pixels with negative values in resulting images were removed using the Clip Signal tool of aXis2000. Finally, these images were colored in blue (for Fe(II)-dominated areas) or red (for Fe(III)-dominated areas), and then overlaid to create composite bicolor images.

2.5. Transmission electron microscopy

The five FIB foils were studied by TEM using a JEOL 2100F equipped with a field emission gun, an ultra-high resolution pole piece, a Gatan energy filter GIF 200, and operating at 200 kV. Selected area electron diffraction (SAED) patterns were performed and analyzed using Image J software. Scanning transmission electron microscopy (STEM)