Geochemical Perspectives Letters

Experimental clues for detecting biosignatures on Mars

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Supplementary Information

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Materials and Methods

Selected Materials

Pure powders of yeast RNA (Sigma-Aldrich) and Mg-smectites synthetised in the lab from a hydrogel of saponite ($Na_{0.4}(Mg_3)(Al_{0.4},Si_{3.60})O_{10}(F_{0.05},OH_{0.95})_2$) were used for the present experiments. The hydrogel was obtained by mixing pure water, hydrofluoric acid, sodium acetate, magnesium acetate tetrahydrate, aluminium acetate basic and silica aerosol. The hydrogel was mixed at room temperature for 3 hours at room temperature (~20 °C) and then introduced in a PTFE-lined stainless steel autoclave. The autoclave was heated at 220 °C for 3 days. After cooling down at room temperature, 5 Ca-saturation cycles were performed for a total duration of ~60 H. The Ca-saturated Mg-smectites were then washed in pure water by centrifugation and dried at 60 °C.

Experimental procedure

We conducted hydrothermal experiments in a 100 mL Parr Reactor © in closed system. For the present study, we experimentally submitted 300 mg of Mg-smectites mixed with 150 mg of RNA to hydrothermal conditions at 200°C in 15 mL of pure bi-distilled water in equilibrium with a CO_2 atmosphere for 7 days at water-vapour pressure (i.e. 15.5 bars at 200 °C). We conducted additional experiments under the same conditions with RNA in absence of Mg-smectites and with Mg-smectites in absence of RNA as controls. Each experiment was performed three times to ensure reproducibility. Experimental residues were washed in pure water five times and dried at 50 °C for ~12 hours.

Analytical measurements

Carbon and nitrogen elemental analysis

Total carbon and nitrogen content were determined using a Flash 2000 Thermo CHNSO elemental analyser operating at ISTeP (France). A mass of 2 to 3 mg of residue was combusted under oxygen/helium flux at 960 °C. N_2, CO_2 released by combustion were separated by a chromatography column and quantified using a thermal conductivity detector. Soil samples were used as standards giving uncertainties at 0.02 wt. % for N and 0.07 wt. % for C.

X-Ray Diffraction measurements

XRD patterns were obtained on a Panalytical X'pert Pro MPD 2 circles operating at IMPMC (Paris, France) at 20 °C and atmospheric pressure. The bulk XRD experiments were performed on powder. The scanning parameters for powder XRD under atmospheric pressure were 0.033°2θ for the step size and 250 s for the counting time per step throughout the 3–65°2θ CuK$\alpha_{1,2}$ angular range. The experiments dedicated to organic matter location were performed on oriented preparations at both atmospheric pressure and under vacuum (3.10^{-4} atmosphere) using an Anton Parr HTK 1200 oven and a temperature monitor TCU1000N coupled to an EDWARDS RV3 pump. The divergence slit, the anti-scatter slit and the two Soller slits were 0.5°, 1°, 0.04° and 0.04 radiant, respectively.

Mid-Infrared (MIR) spectroscopy

Fourier-tranform infrared (FT-IR) spectra have been recorded in the 400-4000 cm^{-1} range with a 4 cm^{-1} resolution using a Nicolet 6700 FTIR spectrometer fitted with a KBr beamsplitter and a DTGS-KBr detector. The powder spectra have been obtained under ambient conditions by averaging 200 scans obtained in attenuated total reflectance (ATR) geometry using a Specac Quest ATR device fitted with a diamond internal reflection element.

Sample preparation for STEM and STXM-XANES experiments

Cryo-ultramicrotome sections (70 nm thick) were prepared for STXM and TEM characterisation using the Leica ultramicrotome available at UMET (Lille, France). Experimental residues were mixed with 0.1 ml of water-ethanol (50/50 $\%_{Vol}$) before being frozen in liquid nitrogen at -160 °C. After cutting, the ultrathin slices of residues were deposited on holey carbon film TEM grids before being exposed to ambient temperature.

STXM-XANES measurements

XANES data were collected using a scanning transmission X-ray microscope on the HERMES STXM beamline (Belkhou *et al*., 2015; Hitchcock, 2018) at the synchrotron SOLEIL. Beamline optical elements were exposed to a continuous flow of pure O_2 to remove carbon contamination. Energy calibration was done before measurements using the well-resolved 3p Rydberg peak of gaseous CO_2 at 294.96 eV. XANES data were extracted from image stacks collected at energy increments of 0.1 eV over the carbon (270–350 eV) absorption range with a dwell time of ≤ 1 ms per pixel to prevent irradiation damage (Wang *et al*., 2009). Alignment of stack images and extraction of XANES spectra were done using the latest version aXis2000 software. The C-XANES spectra shown in the present contribution correspond to homogeneous carbon-rich areas of several hundreds of square nanometres and were normalised to the carbon quantity by integrating the spectra (after subtraction of a power law background) from the pre-edge region up to the mean ionization energy (*e.g*. 282-291.5 eV at the C K edge) following the method proposed and validated by Le Guillou *et al*., 2018).

TEM analyses and post-acquisition data processing

Scanning transmission electron microscopy (STEM) and EDS mapping were performed using a Thermofisher Titan Themis 300 microscope operated at 300 keV, located at the "centre commun de microscopie – CCM" at the university of Lille. Hyperspectral EDS data were obtained using the super-X detector system equipped with four windowless silicon drift detectors. These detectors have a high sensitivity for light elements and allow a high counting rate of the carbon, nitrogen and oxygen X-rays. The probe current was set at 600 pA with a dwell time at 10 µs per pixel.

A key aspect of this work is the post-processing of the hyperspectral data, performed using the Hyperspy python-based package (De La Peña *et al*., 2018). The signal was first denoised using PCA. Then, the EDS spectra at each pixel were fitted by a series of Gaussian functions and a physical model for background/bremsstrahlung. The integrated intensities of the Gaussian functions were used to quantify the compositions thanks to the Cliff-Lorimer method, using experimentally determined k-factors. Absorption correction was taken into account, which is mandatory to correct for the re-absorption within the sample of the carbon, nitrogen and oxygen X-rays. These steps correct for the thickness of the sample. Finally, end-member phases (smectites, phosphates, amorphous silicon oxides, organic compounds) were identified and their spectra used as inputs for linear combination fitting (multiple linear least square fits). Pixels of similar composition were given the same colors scaled as a function of the proportion of each phase.

Supplementary Information References

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