Spectrochimica Acta Part A 83 (2011) 288–296

Contents lists available at SciVerse ScienceDirect

Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy

journal homepage: www.elsevier.com/locate/saa

Investigation of biomolecules trapped in fluid inclusions inside halite crystals by Raman spectroscopy

Kateřina Osterrothová*, Jan Jehlička

Institute of Geochemistry, Mineralogy and Mineral Resources, Faculty of Science, Charles University in Prague, Albertov 6, 128 43 Prague, Czech Republic

ARTICLE INFO

Article history:
Received 9 June 2011
Accepted 19 August 2011

Keywords:
Raman spectroscopy
Evaporites
Fluid inclusions
Amino acids
Astrobiology
Crystallization

ABSTRACT

Raman spectroscopy was tested for the identification of biomolecules (glycine, L-alanine, β-alanine, L-serine, and γ-aminobutyric acid) trapped in fluid inclusions inside halite model crystals. The investigated biomolecules represent important targets for future astrobiological missions. We know from terrestrial conditions that organic molecules and microorganisms can be sealed within fluid inclusions and can survive intact even for hundreds of millions of years. Raman spectroscopy is currently being miniaturized for future extraterrestrial planetary exploration (ExoMars 2018). Raman spectroscopy has shown the ability to detect investigated aminoacids nondestructively without any sample preparation, in short measurement times, and in relatively low concentrations. The number of registered Raman bands of investigated aminoacids and their intensity clearly correlate with the given concentration of biomolecules within fluid inclusions.

1. Introduction

The multidisciplinary field of astrobiology has been growing rapidly in recent years. The major goals of research in the field have been the search for habitable environments both within and outside our solar system, the search for evidence of prebiotic chemistry and life on Mars and other bodies in our solar system, laboratory and field research into the origins and early evolution of life on Earth, and studies of the potential for life to adapt to challenges on Earth and in space. NASA and ESA are heavily focused on a number of upcoming exploration missions (especially the Mars Science Laboratory, with its planned launch in the fall of 2011; ExoMars 2018; and the follow-up Mars Sample Return missions beyond 2020).

The goal of determining whether life ever arose on Mars depends on the question as to whether the Martian environment was ever suitable for life. The confirmation of water [1] on Mars and the discovery of calcium carbonate [2] and perchlorate [3] in the shallow Martian subsurface by the Phoenix lander are among the most important recent findings. If life truly emerged on Mars, it is reasonable to look for it in the areas which may be suitable for preservation of biosignatures. From studies of the fossil records on Earth, we can conclude that these areas usually consist of sedimentary rocks, mainly evaporites (halite [NaCl], gypsum, [$CaSO_4{\cdot}2H_2O$], and anhydrite [$CaSO_4$]) [4]. Evaporitic sedimentary deposits have been observed in numerous areas on Mars [5,6]. With planned sample return missions, the problem of contamination of potential Martian samples with terrestrial organics and microorganisms arises. One solution how to easily face this problem could be nondestructive analysis of fluid inclusions in Martian samples.

Fluid inclusions trapped in minerals work in principle like sealed micro chambers [7]. Inclusions protect their content against any changes in the surrounding environment, and as long as they remain unopened, there is no risk of contaminating of their content. Inclusions represent samples of fluids which existed at some point in the geological history of a rock. They provide clues about the temperature, pressure, density, and composition of the fluids that formed or washed over the rock [8]. Hence, they can trace the early evolution of life on Earth or potentially elsewhere. If an organic material is trapped inside a mineral, it has a better chance to survive the harsh, superoxidizing conditions and high doses of ultraviolet radiation on Mars and stay preserved, even if the samples are collected from the surface or near subsurface [9]. It has been previously reported in many papers that terrestrial halite (NaCl) could contain trapped organic structures and preserve them for even hundreds of millions of years [10–16].

A variety of nondestructive and destructive techniques can be employed in the study of fluid inclusions. Nondestructive methods include visible light microscopy, ultraviolet microscopy, FTIR, microthermometry, Raman microspectroscopy, proton microprobe, electron microprobe, XRD, NMR, and TEM. Destructive analysis is, on the other hand, performed in three basic steps: release, separation, and chemical analysis by methods such as GC, GC–MS, ICP-MS, and SEM [17]. Perhaps the most important nondestructive technique for qualitative, and in some cases even

* Corresponding author. Tel.: +420 221951517; fax: +420 221951496.
E-mail address: osterrot@natur.cuni.cz (K. Osterrothová).

1386-1425/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.saa.2011.08.032

Table 1
List of amino acids used in this study.

Name	Molecular formula	Molecular weight (g/mol)	Solubility in water (mg/L H_2O 25 °C)	Biological occurrence
Glycine	$C_2H_5NO_2$	75.07	2.49	Proteinogenic
L-Alanine	$C_3H_7NO_2$	89.10	1.64	Proteinogenic
β-Alanine	$C_3H_7NO_2$	89.10	5.45	Non-proteinogenic, biological
L-Serine	$C_3H_7NO_3$	105.09	4.25	Proteinogenic
γ-Aminobutyric acid	$C_4H_9NO_2$	103.12	1.3	Non-proteinogenic, biological

quantitative, analyses of fluid inclusions is the employment of Raman microspectroscopy. The major advantage of this method is that it gathers effective information nondestructively, with no sample preparation, in the submicrometer range, and in short measurement times. On account of these properties, Raman spectroscopy was chosen to be a candidate technique for future ExoMars mission to Mars in order to search for the presence of life. Besides Raman spectroscopy, organic biomarkers in fluid inclusions were recently studied successfully by means of destructive methods ToF-SIMS [18,19] and by off-line and on-line crushing followed by GC–MS analysis [20,21].

Several experiments on the preservation potential of evaporites were conducted using laboratory-grown crystals. Adamski et al. [22] experimented with laboratory-grown halite crystals with embedded cells of the bacterium *Pseudomonas aeruginosa*, which were genetically modified to produce green fluorescent protein. By using microscopy techniques, they indicated the long-term survival of microorganisms in fluid inclusions. Fendrihan et al. [23] collected Raman spectra of nine different halophilic strains which were previously embedded in laboratory-made halite crystals. The spectra showed peaks of carotenoid compounds and peaks due to peptide bonds and nucleic acids. Pasteris et al. [24] studied how the dynamics of the crystallization of halite can affect the accumulation and preservation of organic macromolecules present in a starting solution using optical microscopy, atomic force microscopy, and laser-scanning confocal fluorescence microscopy.

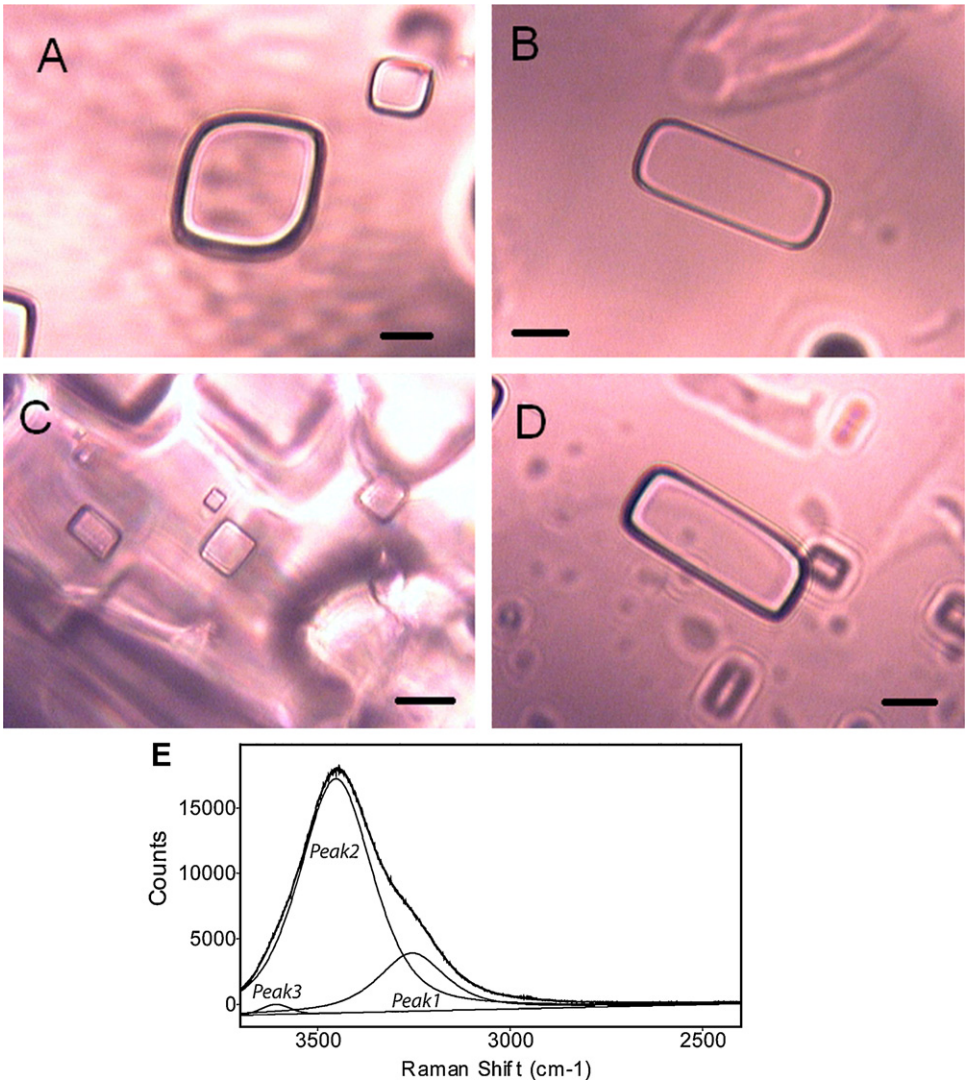

Fig. 1. (A–D) Photomicrographs of fluid inclusions in halite crystals. Scale bar is 20 μm. (E) Deconvolution of a Raman spectrum of NaCl–H_2O solution with three Gaussian–Lorentzian curves. The band positions for the NaCl–H_2O solution are at 3255 cm^{-1} (*Peak* 1), 3452 cm^{-1} (*Peak* 2), and 3609 cm^{-1} (*Peak* 3).