High-resolution imaging of sulfur oxidation states, trace elements, and organic molecules distribution in individual microfossils and contemporary microbial filaments

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Abstract—Owing to the delicate nature of fossil microorganisms and inherent difficulties for discriminating true fossils from artifacts, an important challenge is to extract unequivocal biogenic information from individual microfossils using high-resolution, nondestructive and sensitive techniques. Here, we use combined synchrotron (X-ray microfluorescence, X-ray absorption near-edge structure and infrared microspectroscopies) and particle-induced X-ray emission analyses to image the spatial distribution at a µm-scale of a variety of potential biogenic markers (major and trace elements, C-H bonds, and sulfur-oxidation states) in individual prokaryotic microfossils. In particular, we analyzed iron-oxide fossil filaments of putative biogenic origin encapsulated with amorphous silica from a fragment of an inactive hydrothermal chimney of the East Pacific Rise. In order to test the biogenic origin of the markers studied, we performed the same analyses on filamentous bacteria corresponding most likely to the ε-Proteobacteria, and collected from substrates exposed to a hydrothermal fluid vent at the Mid-Atlantic Ridge. In both types of fossil and contemporary filaments, the occurrence of CH groups and of three sulfur species (sulfate, sulfite, organic S) showing heterogeneous distribution that underline the cytoplasm of individual cells in the case of the present-day filament, suggests that the original microorganisms were actively metabolizing sulfur. These results show the large potential of combining high-resolution synchrotron techniques to analyze individual microfossils for extracting unequivocal biogenic information. Furthermore, they also suggest that cooccurrence of different sulfur oxidation states within single microfossils could constitute a biogenic metabolic marker indicating S-metabolizing activities. Copyright © 2004 Elsevier Ltd

1. INTRODUCTION

Microbially mediated oxidation and/or reduction of sulfur and iron emanating at deep-sea hydrothermal vents in marine environments are known to be important globally (Moyer et al., 1994; Karl, 1995; Reysenbach et al., 2000; Slobodkin et al., 2001). Microorganisms metabolizing different forms of sulfur, and particularly hydrogen sulfide, which is abundantly supplied by fluid emissions, are very diverse. Phylogenetically they range from bacteria to archaea, and they can be hyperthermo-, thermo-, meso- or psychrophilic depending on the temperature niche they are specialized to live in (Stetter, 1996). Many cultivated isolates from these areas oxidize sulfur or thiosulfate, such as many α-, ε- and γ-proteobacteria (Muyzer et al., 1995; Wirsen et al., 1998; Teske et al., 2000), or reduce sulfur, such as several ε-proteobacteria (Campbell et al., 2001; Miroschnichenko et al., 2002). Direct evidence for sulfide-oxidizers excreting elemental sulfur in the form of rigid irregular filaments (Taylor and Wirsen, 1997) have been described from the 9°N deep-sea hydrothermal vents of the East Pacific Rise (Taylor et al., 1999). Similar filaments are produced by marine autotrophic sulfur-oxidizing Arcobacter sp. isolates, also belonging to the ε-proteobacteria (Wirsen et al., 2002). With regards to Fe, recent microbiological studies at the Loihi Sea-mount hydrothermal vents provided the first direct evidence that filamentous oxide morphology were the result of Fe-oxidizing bacteria deposition (Emerson and Moyer, 2002). In addition to cultivated isolates, a wide diversity of ε-proteobacterial lineage have been detected using molecular methods around deep-sea vents, although the type of metabolism they display remains to be determined (Reysenbach et al., 2000; Corre et al., 2001; Longnecker and Reysenbach, 2001; López-Garcia et al., 2002).

Because of their morphologic similarities with modern S- and Fe-oxidizing bacteria producing filamentous sulfur and Fe oxide-encrusted filaments, a putative microbial origin has been proposed for filamentous microfossil remains in fossil hydrothermal vent sites, as well as other ancient environments (Juniper and Fouquet, 1988; Duhig et al., 1992; Little et al., 1999; Trewin and Knoll, 1999; Hofmann and Farmer, 2000; Préat et al., 2000). The oldest reliable microfossil record is that of Rasmussen (2000), who reported pyritic threadlike filaments in deep-sea volcanic rocks that are some 3200 million years old, likely corresponding to thermophilic chemolithotrophs living in subseafloor hydrothermal systems. Morphologic resemblance, however, is not sufficient for discriminating between true microbial fossils and nonbiogenic microstructures, specifically, considering their minute size and incomplete preservation in
For this reason, increasing efforts have been devoted in recent years at developing new approaches for extracting biogenic signal from the geologic record (House et al., 2000; Kempe et al., 2002; Kudryavtsev et al., 2002; Schopf et al., 2002). In this study, a variety of nondestructive and high-resolution synchrotron analyses including synchrotron X-ray microfluorescence (SXRF), micro–X-ray absorption near-edge structure (micro-XANES) and infrared (SIR) microspectroscopies, as well as Particle-Induced X-Ray Emission (PIXE) analyses were performed on fossilized iron-oxide filaments of putative biogenic origin collected from an inactive chimney of the East Pacific Rise (EPR) and microbial filaments collected from an active hydrothermal vent of the Mid-Atlantic Ridge (MAR). The combination of optically discernible morphology, high resolution chemical analysis and distribution maps of sulfur oxidation state and other organic markers (CH groups) on a filament scale provides new tools for investigating further the validity of the biologic origin of some microstructures present in active and ancient hydrothermal systems.

2. MATERIAL AND METHODS

2.1. Samples

The filamentous microfossils were collected from a fragment of an inactive siliceous hydrothermal chimney at a water depth of 2660 m in the northern hump segment at 18°15’ South of the East Pacific Rise (1993 NAUDUR cruise using the Nautil submersible; Fouquet et al., 1994, samples curated by Y. Fouquet). The northern hump segment is characterized by a wide (800 m) and deep (50 m) tectonically active graben with no recent lava and containing abundant inactive hydrothermal chimneys. Magmatic processes occurring on rapid midoceanic accretion zone such as the EPR produce intense and short lived hydrothermal events that lead to ephemeral biologic communities around active hydrothermal sites. Haymon et al. (1993) witnessed, on another part of the EPR, drastic changes in hydrothermal activity within a 2-yr period (comparison of the 1989 ARGO cruise and 1991 AdVenture program), with vent biologic communities developing or disappearing according to shifts in the localization of hydrothermal springs. There-

Fig. 1. (a) Chimney fragment from which the microfossils were obtained (18°15’S, East Pacific Rise), showing a zoning pattern characterized by millimeter-scale layers or pods of different colors (from pale yellow to orange). (b) SXRF spectrum of a Fe-bearing microfossil (arrow) encapsulated in amorphous silica (opal A) from the East Pacific Rise. (c) SXRF spectrum of a MAR bacterial filament collected from a microcolonizer exposed to a fluid emission at the Tour Eiffel chimney (37°17’N; Mid-Atlantic Ridge). White square represents the size of the X-ray beam (2 × 3 μm). The delimited area corresponds to the zone mapped using synchrotron micro-XANES (Fig. 3c) and SIR spectroscopy (Fig. 4). Higher intensity of photon scattering in (b) is due to photon interaction with the opal matrix of the microfossil. Ar peaks on both spectra are due to ambient air.
for, it cannot be excluded that the vent system supporting the microbrial community that resulted in the microfossils used in this study was still active a few years before sample collection. An upper limit for the age of the microfossils is difficult to determine, but because of the vicinity of the collection sites to a fast accretion zone, we can estimate the age of our microfossils to be no greater than a few thousand years. Although EPR samples may not be considered as relevant fossils owing to their relatively young age, they did undergo a fossilization process that resulted in their encapsulation in a mineral matrix. This observation forms the crux of the approach performed here since the mineralized filaments studied can be considered as relevant analogues of older filamentous microfossil remains from ancient hydrothermal systems several million to billion years old.

The iron-silica material examined consist of branching filaments of iron oxide encapsulated with amorphous silica (opal A, Fig. 1a). These are similar to the low-temperature filamentous iron-silica deposits described by Juniper and Fouquet (1988) and Juniper and Sarrazin (1995), which were interpreted to have formed around filamentous microbial mats. However, morphologic observations alone cannot provide unequivocal constraints on the biogenic origin of putative microfossil filaments.

The analyzed bacterial filaments came from an artificial colonization substrate composed of a plastic mesh containing iron fragments that was exposed for 15 days to a fluid emission at the Tour Eiffel chimney in the Lucky Strike site (Mid-Atlantic Ridge; 37°17′N, 1695 m water depth). The colonization device was collected in a close sterile container by the Remote Operated Vehicle Victor during the ATOS cruise in 2001. After recovery, it was opened in a laminar flux chamber on board, and the microbially colonized substrates were fixed in 75% ethanol–2% NaCl and stored at 4°C until use. A molecular survey based on 16S rRNA genes of the bacterial diversity existing on the colonized substrate, showed a large diversity of phylotypes that almost exclusively affiliated to e-proteobacteria (work in progress).

2.2. Sample Preparation

EPR fossil filaments scratched out from a fragment of an inactive chimney were immersed in ethanol. Microbial filaments were extracted directly from the colonized substrates using a syringe. A single drop of each solution was deposited on different 10 μm thick mylar films mounted over an aluminum ring. Drying out the solutions in an evacuated desicator favored the dispersion of individual filaments onto the mylar surfaces. The mylar film was chosen as a support because of its composition (no sulfur or iron could be detected in the film by either PIXE or micro-XANES) and its low X-ray absorption of the soft X-ray beam (2482 eV) used during micro-XANES analysis. Keeping the filaments loosened on the mylar film without a protection cover allowed minimizing further X-ray absorption. Mylar, however, shows several infrared vibrational features within the 1000–1800 cm⁻¹ frequency region where amide bands and CHx bending modes of lipids and proteins displays vibrational fingerprints. For this reason, reference IR spectra were performed on EPR microfossils and contemporary MAR microbial filaments deposited on a ZnS window, which is transparent in the frequency range of interest (see below).

2.3. Synchrotron X-ray microfluorescence (SXRF) and Particle-Induced X-ray Emission (PIXE)

SXRF and PIXE analyses were used to characterize the major and trace elements of individual filaments. Although based on similar physical processes, the photon and proton microprobes display complementary aspects. Of particular relevance is that for a given energy, X-ray production cross-sections are expressed as a function of Z² for SXRF and 1/Z² for PIXE. Z corresponding to the atomic number of the target element. As a consequence, the nuclear microprobe is better-suited for light element detection (Z < 22) whereas SXRF analysis are highly sensitive to heavy elements, with detection limits down to the ppm level for elements with absorption edge close to the incident beam energy (17 keV, see below). PIXE is characterized by a higher spatial resolution (1 × 1 μm) than SXRF technique (2 × 3 μm) and is therefore best-adapted for mapping purposes at the scale of our samples. In contrast, owing to their damaging character, proton particles are not adapted to fragile organic structures, and SXRF is required for analyzing bacterial filaments.

SXRF measurements were performed on the undulator beamline ID22 of the European Synchrotron Radiation Facility (ESRF). The incident radiation was monochromatized by means of a double crystal fixed-exit monochromator ([111] plane of Si). A crossed mirror system based on a Kirkpatrick Baez design ensured the focusing of monochromated X-rays at 17 keV, with a spatial resolution of 2 × 3 μm at a flux of 3.10⁻¹² ph/s. Spectra were recorded with an acquisition time of 300 s. Owing to the relative large size of the beam, compared to the filament diameter (Figs. 1b,c), no mapping was performed using SXRF.

PIXE analysis allowed the distribution of transition metals forming the internal zone of the filamentous microfossils to be mapped. Analyses were performed at the Pierre-Süe Laboratory nuclear microprobe (Khodja et al., 2001). A proton beam of 2.5 MeV at 400 pA was used for a total charge of ~3 μC. The beam was focused to ~1 × 1 μm and scanned over large areas of the samples.

2.4. Synchrotron Micro-XANES

Micro-XANES spectroscopy was used to examine the relative amount and spatial distribution of the different oxidation states of sulfur present in a single filament. Micro-XANES spectra and images were obtained using beamline ID21 (Susini et al., 2002) at the ESRF. The structure of the S K-edge was scanned in the near edge region. Incident beam energies from 20 eV below the main absorption edge energy of sulfate (2482 eV for S⁶⁻) to 20 eV above the main edge were used. The microscope used a Fresnel zone-plate as a focusing lens and delivered a microbeam of 0.8 × 0.8 μm² with a photon flux of ~10⁸ photons/s (David et al., 2000). Recorded micro-XANES spectra were calibrated and compared with spectra of pure standard products (elemental sulfur, sulfides: FeS, FeS₂, Fe₃S₄, S, CuS, (Cu,Fe)S₂, CuFeS₂, ZnS, (Zn,Fe)S, sulfite: Na₂SO₃, sulfates: Fe⁴⁺SO₄, Fe⁶⁺(SO₄)₂, CuSO₄, ZnSO₄, NiSO₄, CaSO₄, BaSO₄, amino acids: methionine, cystine, cysteine and SH-bearing glutathion) the most relevant of which are
Fig. 3. PIXE and micro-XANES results. (a) PIXE spectrum and elemental distribution of a microfossil. Note the good correspondence between the internal zone of the filament (arrow) and the distribution of Fe, Cu (and Zn, not shown here), and dissemination of Cl on exterior of the siliceous walls. White square represents the size of the X-ray beam (1 $\times$ 1 $\mu$m).

(b) Typical synchrotron micro-XANES spectra of sulfur recorded in a MAR bacterial filament (red) and EPR microfossil.
shown in Fig. 3b. The standards were analyzed during the same experimental session to optimize the calibration procedure and interpretation of the X-ray spectra.

2.5. Photoreduction during Micro-XANES Experiments

Interaction of intense X-ray photon beam with organic and inorganic matrices is known to be responsible for the photoreduction of the more oxidized compounds into reduced species (F. Farges, private communication). In the present case, the problem lies in identifying the potential of reducing sulfates (S\(^{6+}\)) into less oxidized species such as sulfites (S\(^{5+}\)) or sulfides (S\(^{2-}\)). To test whether photoreduction occurs during X-ray irradiation of the sample studied, we performed a series of micro-XANES acquisition on a bacterial filament exposed to different irradiation rates (Fig. 2). The three absorption spectra shown in Fig. 2 correspond to successive acquisitions on the same spot after 0, 12 and 60 min of irradiation of the sample using an incident energy of 2500 eV. Figure 2 shows that the two main peaks at 2473 and 2482 eV remain relatively unmodified in the different spectrum, but that a low intensity sulfide peak at 2470 eV arises in the 12 and 60 min spectra. The absence of the 2470 eV peak in the spectrum obtained on the nonirradiated specimen (t = 0) indicates that photoreduction does occur in the sample analyzed. However, this reduction remains very limited owing to the low quantity of sulfides produced. Determining which sulfur compound was reduced to produce sulfide remains difficult since the differences of the 2473 and 2482 eV peak intensities in the three spectra remain within statistical error.

2.6. Synchrotron Infrared Microspectroscopy

SIR microspectroscopy was used to investigate potential vibrational modes associated with the presence of proteins, lipids and nucleic acids in the single filaments. SIR microanalysis were performed on the bending magnet SAS at Super-ACO (beamline MIRAGE) at the Laboratoire pour l’Utilisation du Rayonnement Electromagnétique (LURE) (Polack et al., 1999). The synchrotron beam is extracted from a bending magnet, through two ZnSe windows and coupled to a Magna-IR 560 FTIR bench spectrometer to which a Nic-Plan IR microscope (Thermo-Nicolet) is attached. All spectra were obtained after 256 accumulations at 8 cm\(^{-1}\) resolution, and with a 6 × 6 μm spatial resolution.

3. RESULTS AND DISCUSSION

Linking biogenic signals to individual microfossil structures is urgently needed to unambiguously assess the biologic nature of ancient microfossils. Synchrotron-based and PIXE techniques allow high-resolution and nondestructive chemical imaging of micron scale objects embedded in complex geological matrices (Philippot et al., 2000, 2001; Ménez et al., 2002; Foriel et al., 2003). Accordingly, we addressed two major issues in this work: 1) whether these techniques could be successfully applied to element mapping at individual filamentous bacteria and microfossil scale, and 2) whether mapping of particular elements or element oxidation states could constitute a bio-marker in geological material.

To answer these questions, we first performed a chemical map of a fossil filament fragment from an inactive chimney (Fig. 1a) using PIXE technique (Figure 3a). The analysis revealed that Fe, Cu and Zn are localized in the filament core, in agreement with textural observations. In contrast, Cl, Ca and K occur as disseminated zones located on the exterior siliceous walls, thus arguing for an externally-derived contamination from seawater. The distribution of Fe, Cu and Zn in the internal area of the opal structure once occupied by a bacterial filament suggests that it contained these elements and/or induced their precipitation on its cell surface (Fortin et al., 1998) either during its lifetime or during the fossilization process. In fact, Fe, Cu and Zn can be used as cofactors by living cells, and Fe-based microbial metabolisms are important in hydrothermal regions. Several deep-sea vent species are able to reduce Fe(III), whereas others can oxidize Fe(II) forming iron-oxide crusts (Emerson and Moyer, 2002).

Although the PIXE technique is well adapted for characterizing major element distribution on a microfossil scale, the highly intense and nondestructive photon source provided by the ESRF is required for determining the trace element content of individual microfossil and fragile bacterial filaments. SXRF analysis revealed the presence of S, Cl, K, Ca, Mn, Fe, Cu, Zn, Pb, Br and Sr in both the EPR microfossil and the MAR bacterial filaments (Figs. 1b.c). In addition to these, Cr and Ni have been detected in the microfossil (Fig. 1b), and Tl, Se and Rb in the bacterial filament (Fig. 1c). Owing to the weak excitation cross-sections for light elements using an incident energy of 17 keV and the high absorption of soft X-rays by the siliceous envelope, only a weak X-ray peak of sulfur was identified in the fossil filament. In the case of elements such as S, Se, Fe, Mn and possibly other transition metals like Ni, Cr, Cu and Zn, a biologic source should be considered, since they can be used as enzyme cofactors or as an energy source in redox reactions. In contrast, Cl, K, Ca, Ba, Br, Pb, Rb and Sr are major constituents of seawater and hydrothermal fluids and, therefore, they most likely correspond to external contaminants coating the external surfaces. The origin and role of thallium remains enigmatic. Elemental concentration estimates, corrected for X-ray absorption of the incident and fluorescent beam by the host silica envelope in the case of the fossil filament (Philippot et al., 1998), range between 3000–50,000 ppm Fe, 300–5500 ppm S, 10–540 ppm Ca, Mn, Cr, Ni, Cu and Zn, and 10–28,000 ppm Cl. In the bacterial filament, other concentration estimates yielded 290 ppm Sr, 35 ppm Pb and less than 10 ppm for Br and Se. In the microfossil, Br, Sr and Pb are less than 1 ppm. The large variations in calculated concentration values reflect the heterogeneous elemental distribution on a filament scale and therefore, should be considered with caution.

The results obtained by SXRF and PIXE demonstrate, first, that these techniques are suitable for determining the trace element distribution on a filament scale, and second, that an internal element signal remains in the silicified fossils that can be reasonably attributed to a microbial-derived origin and not to contamination, as indicated by the differential distribution of transition metals within the fossil core. This prompted us to
further explore in these fossils the presence of sulfur in its different oxidation states, since sulfur-based metabolisms are especially relevant in hydrothermal systems and could perhaps leave traces recording past biologic activity.

Micro-XANES results are, to our knowledge, the first chemical imaging of sulfur redox distribution on a bacterial filament scale. The micro-XANES spectra recorded on individual microfossils and, as a control, on a living bacterial filament showed similar features (Fig. 3b): three main types of sulfur with X-ray peaks at 2473, 2478 and 2482 eV are found. The 2473 and 2482 eV peaks were ubiquitous in all samples, whereas 2478 eV was found more sporadically. Upon comparison with standards XANES spectra (Fig. 3b and Sarret et al., 1999; Bonnin-Mosbah et al., 2002; Métrich et al., 2002; Cuif et al., 2003), these peaks can be attributed to organic sulfur (S-C2.5) and therefore, the occurrence of fossil sulfates and sulfates is necessary to quantitatively evaluate the respective contribution of these compounds. A photoreduction origin cannot be excluded for these low intensity peaks (see section 2.5) and therefore, the occurrence of fossil sulfate cannot be evaluated. Simulation of XANES spectra by adapting the full multiple-scattering theory (Bonnin-Mosbah et al., 2002) to sulfur suggests that the absorption of the 2921 cm−1 band observed on the microfossil and the bacterial filament as CHx stretching mode. In the MAR bacterial filament spectra absorption bands at 1542–1650 cm−1, along with broad peak around 3300 cm−1, correspond to amide N-H vibration. In the EPR microfossil, the peaks present in the amide absorption region (1607 cm−1 on mylar film and 1630 cm−1 on ZnS) are not characteristic of amide I + amide II absorption bands. Therefore, whether amide is preserved in the microfossil is uncertain and we cannot conclude on the nature of the molecules responsible for the IR features in the EPR microfossil. Since analyses were not performed in a dry state, it can not be excluded that features observed on the microfossil spectra (peaks at 1607–1630 cm−1) are due to a water film. These results are similar to those observed on different populations of cultivated microorganisms (Naumann, 1998, 2002). Accordingly, it is proposed that the SIR results confirm that organic molecules have been at least partially preserved in the EPR microfossil (as C-H) and are still present in the MAR bacterial filament as C-H and amide. Figure 4 shows also an IR map of the MAR bacterial filament performed at 2921 cm−1. Even though the 2–3 μm wide filament can only be poorly imaged by IR spectroscopy (IR spatial resolution is ~6 μm), recognition of the absorption of the 2921 cm−1 centered on the filament confirms that C-H are indeed present in the filament. Because the size of the filamentous microfossil analyzed is in the order of the lateral resolution of the beam, the infrared absorption is due to the presence of organic material and is not due to a water film. Organic contamination is an extremely unlikely origin for CH− because it would not result in a distribution that matches the filament morphology. Additionally, numerous spectra collected outside the filament using either mylar or ZnS substrate never showed significant absorption in the 2850–2935 cm−1 region (dashed line in Fig. 4).

Overall, recognition that the CH− groups (and amide for the bacterial filament) maps overlay sulfur distribution of the living bacterial filament further supports the interpretation that the sulfur signal is of biogenic origin. The sulfur redox distribution coupled with the CH− group signal are not limited to the bacterial filament, but are also found in the filamentous microfossils. This observation demonstrates that filamentous Fe-bearing microstructures embedded in silica from an inactive
hydrothermal chimney (EPR) are of biogenic origin. It also shows that organic molecules signal can be remarkably resistant to the fossilization process.

4. CONCLUSIONS

Taken together, our results demonstrate the power of synchrotron-based techniques for imaging fossil and living cell components at a single cell scale. Their application to fossil compared to living systems can be very helpful to identify potential biogenic signatures, such as microscale mapping of elements linked to biologic activity that may leave traces during fossilization. It can also help to establish authentication criteria by discriminating, in the case of siliceous fossils, if element distribution occurs inside microfossil structures and is therefore associated to the original microorganism, or if it is a product of later contamination on external siliceous layers. Our micro-XANES analysis showing the coexistence of different S oxidation states and the presence of sulfur-bearing organic molecules in microfossils and bacterial filaments (most likely \( \varepsilon \)-proteobacteria metabolizing S from vent fluids) from hydrothermal systems suggests that distribution of sulfur species could constitute a biogenic signature and serve to track a particular metabolic activity. Although very promising, further work and controls will be required to determine whether sulfur species mapping can be used as unambiguous biogenic marker for its application to older fossils.

A final conclusion is that the combination of high-resolution and nondestructive synchrotron analysis combining SXRF, sulfur K-edge micro-XANES and SIR microspectroscopy techniques offers new complementary tools to study bacterial fossilization. If our results, obtained from recent objects, can be gradually extended to older fossils, they may open new perspectives for the interpretation of the origin of some microstructures found in ancient Archaean (2.5 to 4.0 billion years old) fossilized systems, since the oldest biogenic traces, including carbon-sulfur stringers (Nisbet and Fowler, 1999), pyritic filaments (Rasmussen, 2000), and sulfide-rich bands with characteristics biogenic isotopic signatures (Grassineau et al., 1999; Shen et al., 2001) are thought to record the activity of autotrophic thermophiles living in hydrothermal systems.

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Fig. 4. Synchrotron micro-IR spectra of the EPR microfossil and MAR bacterial filament deposited on mylar (upper diagram; same samples as those analyzed for sulfur oxidation states in Fig. 3c) and on ZnS substrate (lower diagram). In both spectra, the dashed line correspond to the SIR spectrum recorded on mylar and ZnS. Associated map shows the distribution of CH- groups at 2921 cm\(^{-1}\) in the bacterial filament shown in inset. Black square on the bacterial filament micrograph represents the spatial resolution of the IR beam. (*) refers to the peak used for the mapping and shown in the enlarged region of the SIR spectra obtained on mylar.


