

Insight into bacteria: magnetosomes chains under photoemission electron and scanning electron microscopes

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ABSTRACT

Magnetotactic bacteria (MTB) are forming magnetosome chains consisting of greigite or iron oxides, which are considered as perspective material. We report on the first studies of MTB by means of photoemission electron microscopy (PEEM) and scanning electron microscopy with energy dispersive x-ray spectrometry (SEM-EDX). We present two preparation methods for *Magnetospirillum magnetotacticum* MS-1, yielding intact bacteria with their in vivo structure. We will demonstrate that PEEM is suitable to investigate the magnetosome chains. Since each point of the entire image is acquired simultaneously and without scanning, this could become beneficial for studies of dynamic processes. Further, we report on the first SEM images in which individual magnetosomes can be directly depicted within intact MTB. By performing SEM-EDX measurements we are able to confirm the composition of the magnetosomes.

Keywords: SEM, XAS, EDX, PEEM, magnetosomes

1 INTRODUCTION

Magnetotactic bacteria (MTB), like *Magnetospirillum magnetotacticum* MS-1 [1] studied in this work, form chains of membrane encapsulated particles called magnetosomes [2, 3]. These chains consist of greigite (Fe_3S_4) [4] or magnetite (Fe_3O_4) [5], depending on the species of bacteria. With the help of these magnetosomes an oriented navigation of the bacteria along Earth's magnetic field becomes possible [6]. For the bacteria this is very important, since they seek the oxic-anoxic transition zone (OATZ) which is their optimal living environment [7].

MTB are considered as a perspective material for various applications [8, 9]. Beside the pure magnetosomes, the entire bacteria could be used as a controllable

and MRI-trackable propulsion and steering system for medical nano-robots operating in the human microvasculature, for instance in cancer therapy [10].

So far, various techniques have been applied to study MTB or extracted magnetosomes. These are for instance, magnetic force microscopy [11], low-temperature SQUID magnetometry [12], and coercivity deconvolution [13]. Nevertheless, two techniques providing spatial, chemical, and even magnetic information were not applied on intact MTB in order to directly access the magnetosome chains. These two techniques are, photoemission electron microscopy (PEEM) and scanning electron microscopy in combination with energy dispersive x-ray spectrometry (SEM-EDX). In the only known SEM images of intact MTB with hints of a chains structure, the magnetosome chain is just identified by bulges of the cell envelope, without the possibility to identify individual magnetosomes [14].

A preparation, combined with a sampling method to study MTB on a more complex substrate than the normally used grids would be of particular interest for further investigations. PEEM could be a suitable method for this task. With a sufficient resolution, it can directly image the magnetization of magnetosomes. In the future, studies on MTB's response to external stimulations might be possible by combining PEEM's time resolution with more complex substrates. This requires a preparation method which yields intact bacteria on a solid substrate. Further, it must be clarified in how far this technique is suitable for studies of magnetosome chains within intact MTB.

2 TECHNIQUE & SETUP

PEEM uses electrons excited by the photoelectric effect to image the sample surface [15]. There are two operation modes distinguishable by the photon energy

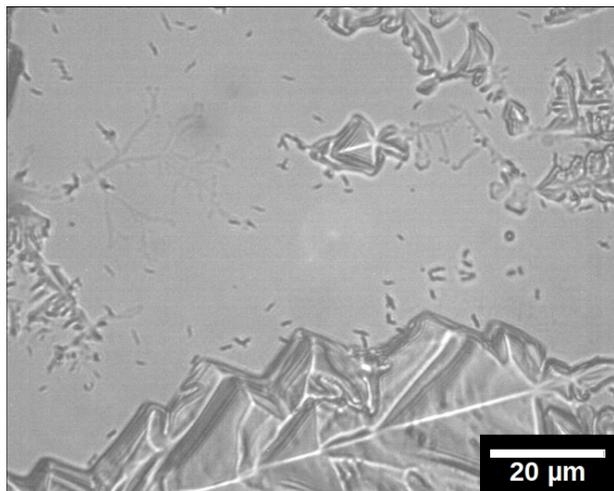


Figure 1: Light microscope picture of a dried sample prior to the final rinsing procedure. The MTB are clearly visible as small, dark rods. Remaining salt crystals can still be found, ranging from large crystals (lower edge) down to fine, branch like structures (e.g. top, left quarter).

used to excite these electrons. The “threshold-emission mode” yields a high spatial resolution by using rather low photon energies to excite electrons out of the valence band. The contrast in this mode is dominated by differences in the work function, as well as topographic features. In the “high-energy mode” secondary electrons are used to image the sample. Here, spatial resolved x-ray absorption spectra (XAS) become possible by recording the electron yield of a specific region of interest (ROI) as a function of the photon energy [15, 16, 17]. Hence, PEEM combines microscopic and spectroscopic techniques providing spatially and spectrally resolved information of the sample.

The PEEM measurements shown here were performed by using a STAIB-PEEM-350 in threshold-emission mode ($h\nu = 4.9\text{ eV}$), and an Omicron-Focus PEEM at the SGM-beamline 4-ID-C at the Advanced Photon Source (APS), Argonne, providing circularly polarized synchrotron radiation. A SEM Quanta 200 FEG from FEI, and a Bruker EDX spectrometer Quantax 400 SDD X-Flash 4010, with an energy resolution of 123 eV at Mn- K_{α} were used for the SEM-EDX measurements.

3 SAMPLE PREPARATION

The cultures of MS-1 are exposed to an oxygen atmosphere, in order to start the natural migration towards the OATZ. After the self-enrichment of the MTB, the mixture of bacteria and nutrient solution is extracted out of the OATZ and handled according to one of the following two preparation procedures.

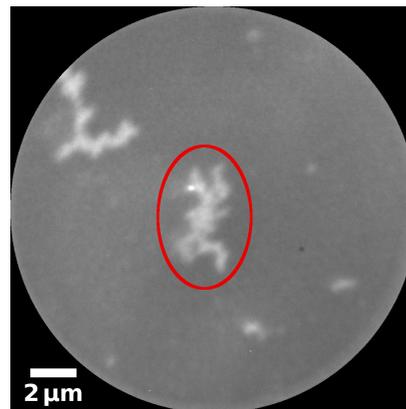


Figure 2: PEEM-image of a sample prepared at the APS ($h\nu = 703\text{ eV}$). A bunch of bacteria is clearly visible in the center (red ellipse). The surrounding silicon surface is nearly residual free.

Method one is rather time-consuming, however, yields a high bacteria density. Here, the mixture is repeatedly centrifuged and resuspended using a phosphate buffered saline. This washed, bacteria rich solution is further diluted with purified water and dried on a sample carrier. The used sample carriers were either glass microscope slides, or silicon wafers for light microscopy, or PEEM and SEM-EDX, respectively. The resulting sample surface is shown in Figure 1. There are still regions with a high amount of salt crystals present. Since these would cause a changing of the sample during PEEM and SEM measurements, the dried sample is finally rinsed with purified water.

The second method is simpler and faster, at the expense of bacteria density. Here, the extracted mixture is directly diluted by adding purified water. The diluted mixture is then applied onto a sample carrier and dried, as in the previous preparation method.

4 RESULTS

PEEM measurements in threshold-emission mode result in distortion free images of MTB in their in vivo structure [16]. Hence, the sample surface features negligible salt residuals, as larger salt residuals would cause a localized charging of the surface, resulting in image distortions. Therefore, our preparation techniques yield pristine sample surfaces with intact bacteria most suitable for PEEM and SEM measurements.

XAS measurements were performed at the APS by using circularly polarized light, in order to determine the accessibility of the magnetosome chains within the intact bacteria. Figure 2 shows the sample region, used for these measurements. A bunch of bacteria is located in the image center, marked by a red ellipse. The sur-

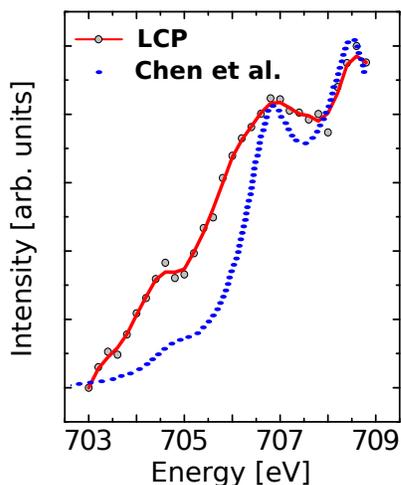


Figure 3: PEEM-XAS spectrum of the MTB bunch, shown in Figure 2, recorded with left circularly polarized light (LCP). The features in the calculations for bulk magnetite by Chen et al. [18] are in excellent agreement with the measurements.

rounding sample surface is nearly clean, except an additional bunch of bacteria in the top left corner.

Although the magnetosomes are only a small fraction for the entire bacterium, PEEM still yields a clear XAS signal through the cell wall, as Figure 3 shows. This spectrum is solely extracted from the central bacteria bunch in Figure 2. The other bunch drifts out of the field of view during the measurements and can therefore not be used. Three features at photon energies of 704.6 eV, 706.8 eV, and 708.6 eV are clearly visible within the spectrum. Their positions are in excellent agreement with calculations done by Chen et al. [18]. There, the XAS of bulk magnetite was calculated for an excitation by left circularly polarized light. The position of these three features is strongly related to the magnetic properties, rather than the chemical composition of the sample. Therefore, we can directly access the magnetic properties of the magnetosome chain.

Beside the PEEM measurements, SEM and SEM-EDX measurements were conducted as well. Since SEM has similar requirements regarding the sample surface as PEEM, the identical preparation procedures were used. Figure 4 shows an example of the SEM images achieved in this way. The bacteria are surrounded by a nearly clean Si surface. Slightly larger solution residuals persist only in the area between the three MTB. Further, the magnetosome chain can be clearly identified within the bacterium. Even the individual magnetosomes are distinguishable through the cell wall. In this manner, even small alterations like the deformed magnetosome in the center of the chain (white arrow) can be easily examined.

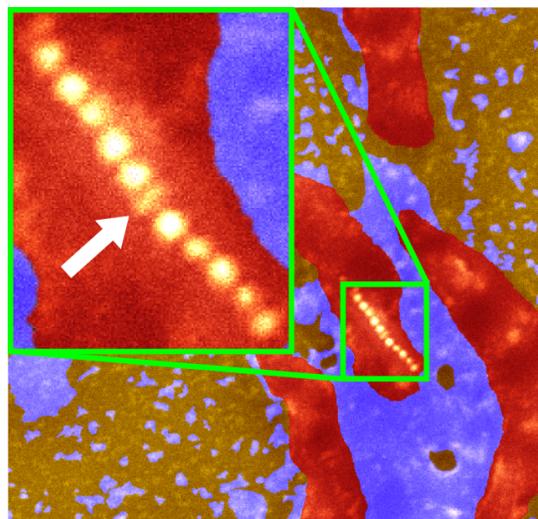


Figure 4: False color SEM image of MS-1 (red) on Si (gold), surrounded by solution residuals (blue). The magnetosome chain is clearly visible (inset). Even small alterations in the individual magnetosome's shape are clearly visible (white arrow).

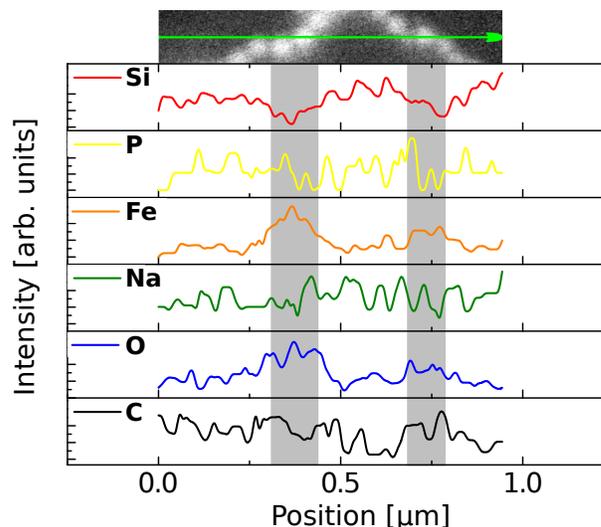


Figure 5: SEM-EDX scans along the green line in the SEM image (inset), crossing two magnetosome chains. The modulation of the Fe and O signals confirm that the magnetosomes consist of magnetite, rather than greigite. The inverse variation of the Si signal, compared to the Fe and O signals, can be explained by a lower sampling depth caused by the magnetite.

In order to verify the magnetosome's composition for this species, SEM-EDX measurements were performed, too. Figure 5 shows the results of such an SEM-EDX line scan crossing two magnetosome chains. Although, phosphorus, sodium, and carbon show some variation, these are in no way correlated to the position of the magnetosomes. The variation of these signals is rather an effect of other parts of the bacterium cell, beside the magnetosome chain. However, the iron as well as the oxygen signal correlates nicely with the crossing points of line scan and magnetosome chains. This clearly indicates that the magnetosomes consist of an iron oxide and not of an iron sulfide. Therefore, MS-1 forms magnetite magnetosomes rather than greigite magnetosomes. In addition to iron and oxygen, the silicon signal modulates at the points where magnetosome chains and line scan cross, too. Here, the intensity of the silicon signal drops, in contrast to the rising iron and oxygen signals at the same positions. This can be explained by SEM-EDX's reduced sampling depth caused by the presence of the heavy iron in the magnetosomes' magnetite. Thus, less of the silicon substrate is sampled in these regions.

Further, SEM-EDX spectra of entire magnetosome chains feature no sulfur K_{α} -signal at 2.3 keV. This is an additional proof that the magnetosomes do not consist of any iron sulfide, like greigite.

5 CONCLUSION

In conclusion we could show two preparation methods as well as two new experimental methods, which are able to give a new insight into interesting bacteria with a wide range of applications.

PEEM was demonstrated as a new method for accessing magnetosome chains within intact MTB. This method not only yields chemical, but also magnetic information from within the bacterium. Since PEEM acquires the entire field of view simultaneously, unlike scanning techniques, it is especially suitable for studies of dynamic processes. This might be, for instance, the response of the fixed MTB to changing magnetic fields.

Our SEM-EDX measurements demonstrate that our preparation method yields samples, which need no coating by gold or any other metal to ensure a sufficient conductivity. Therefore, we can access the inner structure of MTB by SEM, which was previously not possible [14]. This is of particular interest for the characterization of newly discovered MTB species. Thus, the composition of magnetosomes can be determined by using a technique relatively easy to handle and commonly used in many laboratories. In contrast to other methods, this does not require a destruction of the bacteria to extract the magnetosomes. Therewith, the same samples can be used further on, for studies of the chain structure, for example.

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