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Characterization of Magnetosomes After Exposure to the Effect of the Sonication and Ultracentrifugation

M. Molčan[a,*], A. Hashim[a], J. Kováč[a], M. Rajňák[a], P. Kopčanský[a], M. Makowski[b,c], H. Gojzewski[b,d], M. Molokáč[e], L. Hvizdák[e], M. Timko[a]

[a]Institute of Experimental Physics, SAS, Watsonova 47, 040 01 Kosice, Slovakia

[b]Institute of Physics, Poznan University of Technology, Nieszawska 13A, 60-965 Poznań, Poland

[c]Max Planck Institute for Polymer Research, Ackermannweg 10, 55-128 Mainz, Germany

[d]Max Planck Institute of Colloids and Interfaces, Department of Interfaces, Wissenschaftspark Potsdam-Golm, Am Mühlenberg 1 OT Golm, 14476 Potsdam, Germany

[e]Institute of Geotourism, Technical University of Košice, Letná 9, 042 00 Košice, Slovakia

Magnetosomes are intracellular organelles of widespread aquatic microorganisms called Magnetotactic bacteria. At present they are under investigation especially in biomedical applications. This ability depends on the presence of intracellular magnetosomes which are composed of two parts: first, nanometer-sized magnetite (Fe_3O_4) or greigite (Fe_3S_4) crystals (magnetosome crystal), depending on the bacterial species; and second, the bilayer membrane surrounding the crystal (magnetosome membrane). The magnetosomes were prepared by biomineralization process of magnetotactic bacteria Magnetospirillum Magnetotacticum sp. AMB-1. The isolated magnetosome chains (sample M) were centrifugated at speed of 100000 rpm for 4 hours (sample UM) and sonicated at power of 120 W for 3 hours (sample SM), respectively. The prepared suspensions were investigated with respect to morphological, structural and magnetic properties. The results from scanning electron microscopy showed that isolated chains of magnetosomes were partially broken to smaller ones after ultracentrifugation. On the other hand the application of the sonication process caused the formation of individual magnetosomes (unordered in chain). These results were confirmed by coercivity and magnetization saturation measurements.

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1. Introduction

The magnetosomes are monodomain, well-crystallized nanoparticles surrounded by a lipidic membrane with the unique property of being usually arranged in chains. The mono-domain character of studied samples was confirmed by HRTEM, given in the paper [1]. They were synthesized by a group of bacteria, called magnetotactic bacteria, which use them as a compass to navigate in the direction of the Earth magnetic field [2].

Magnetosomes are characterized by narrow grain-size distributions (30 – 120 nm), distinct species-specific crystal morphology, chemical purity, and arrangement in single or multiple linear chains [3, 4]. After isolation from these bacteria, those chains tend to form closed loops so as to minimize their magnetic stray field energy [5, 6]. These procedures leave the surrounding membrane intact and magnetosome preparations are apparently free of contaminating material. Owing to the presence of the enveloping membrane, the isolated magnetosome particles form stable, well-dispersed suspensions.

2. Materials and methods

Bacterial magnetosomes were synthesized by the biomineralization process of magnetotactic bacteria Magnetospirillum strain AMB-1. Bacteria produce magnetite – Fe_3O_4 particles. The process of isolation of individual magnetosomes chains from the bacteria body consists of several steps: sonication, centrifugation and magnetic decantation [7]. For the purpose to obtain the individual magnetosomes, the isolated magnetosome chains (sample M) were ultracentrifuged at speed of 100000 rpm for 4 hours at 4 °C (sample UM) and ultracentrifugated at 100000 rpm for 3 h and sonicated at power of 120 W for 3 hours (sample SM), respectively.

For Atomic Force Microscopy (AFM) the samples were prepared by spin coating. Magnetosomes diluted in HEPES solution (1 mM HEPES solution in double purified MilliQ water) were deposited on polished silicon wafers at rotation time of 60 s and rotation speed of 1500 rpm. Prior the depositions, the silicon wafers were chemically treated by Piranha solution, later flushed by pure water and dried under a nitrogen stream. After deposition the layer of magnetosomes was left to dry completely at room temperature. AFM experiments were performed by NanoWizard II JPK. The AFM images were obtained in the tapping mode with a standard AFM tip (Olympus, model OMCLAC 160 TS, nominal tip radius < 10 nm). All measurements were done in air (relative humidity in the range of 25-40%) at room temperature, in a low-noise acoustic chamber.

The micrographs of magnetosomes were obtained by Transmission Electron Microscopy (TEM) FEI Tecnai F20 S-TWIN Philips. TEM was operated using the accelerating voltage of 200 kV in a bright field mode. A

*corresponding author; e-mail: molcan@saske.sk

drop of the same solutions as for specimens for AFM investigations was placed (drop coating) on 300-mesh carbon-coated copper grids. The solution (solvent) was left to dry completely at room temperature. Such prepared specimens were immediately used for imaging.

Magnetization measurements of the prepared magnetosomes suspension were carried out by SQUID magnetometer of Quantum Design at room temperature.

3. Results and discussion

Figure 1 shows scanning images (TEM and AFM) of three samples of magnetosomes: (I) not influenced by separation method (standard magnetosomes sample, i.e. magnetosomes of long chains), (II) after centrifugation procedure (100000 rpm, 8 h), and (III) after centrifugation procedure (100000 rpm, 12 h) including sonication (120 W, 3 h).

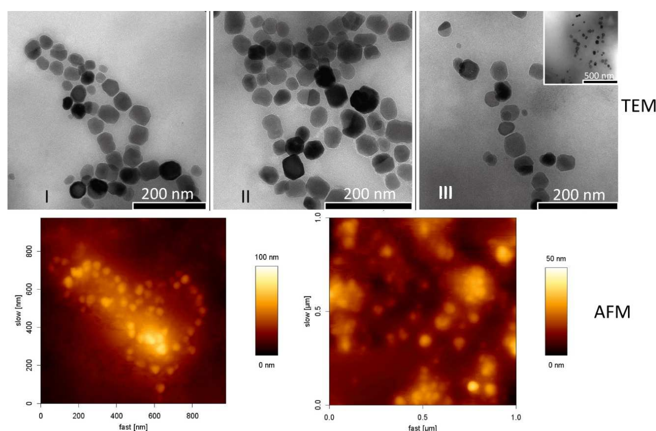

Fig. 1. TEM and AFM images of magnetosomes, deposited on solid surfaces (TEM – drop coating on carbon-coated copper grid, AFM – spin coating on Si wafer): I (MP), II (UM) and III (SM), (inset shows a large scan area of the same sample – the result indicates a strong impact of the separation procedure that uses sonication treatment).

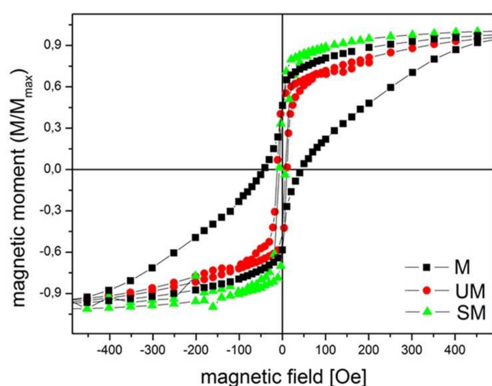

Fig. 2. Hysteresis loops for sample M, UM and SM.

Sample I shows characteristic features for magnetosomes, i.e. long chains. Due to centrifugation at high rotation speed of 100000 rpm the magnetosomes' chains (sample II) are shorter than in case of sample I. They are also aggregated, since they have more freedom to move (interaction forces start to play a role, in contrast to reduction of the magnetic momentum of each chain). Individual, single magnetosomes are also visible, but rare. Sample III presents almost no long chains (see inset also), but small groups of a few magnetosomes (not ordered) and certain amount of single magnetosomes. This result indicates that the desired separation force has been greatly exceeded after sonication. We have also observed that the centrifugation at the same rotation speed, but different procedure time (e.g. 4 h vs. 12 h), has minor effect on separation of magnetosomes' chains, whereas time of sonication has a significant impact on separation (e.g. 2 h vs. 3 h) (data not shown). The average size of the magnetosomes is described in the paper [8].

The M–H hysteresis loops (Fig. 2) measured at room temperature show a ferromagnetic property of all samples with the same saturation magnetization of $Ms = 2.1$ emu·g^{-1} and coercive field of 41 Oe, 12 Oe and 7 Oe for sample M, UM and SM, respectively. These results correspond to the results obtained from microscopy measurements. The orderly arranged magnetosomes in the chains have strong interparticle dipolar interactions, so exhibiting a higher coercivity than the separate magnetite nanoparticles. It is the main reason why for ultracentrifugated and sonicated sample, containing partly individual particles, the coercive force is lower as for the isolated sample only.

4. Conclusions

The application of ultracentrifugation and sonication processes to isolated magnetosomes arranged in chains allowed us to prepare shorter chains and partly the individual magnetosomes, which was confirmed by TEM and AFM microscopy measurements and by obtained values of the coercive field.

Acknowledgments

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