FORMATION AND MAGNETIC PROPERTIES OF MAGNETOSOMES

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Abstract

Formation of biological magnetic nanoparticles (magnetosomes) was achieved by a mineralization process with biological control over the accumulation of iron and the deposition of the mineral particle with specific size and orientation within a membrane vesicle at specific locations in the cell of magnetotactic bacteria Magnetospirillum sp. AMB-1. The mean diameter of isolated magnetosomes was estimated from TEM to be as 34 nm. The saturation magnetization of the magnetosomes was estimated to be 62 emu/g what is smaller than for chemically synthesized magnetite 75emu/g at room temperature due to presence of nonmagnetic organic layer. The curve of field dependence of magnetization at 293 K exhibited the remanence of 21 emu/g and coercivity of 185 Oe what is connected with fact that the mean diameter (34 nm) is larger than critical size for transition from superparamagnetic to ferromagnetic behaviour.

Introduction

Magnetic nanoparticles in diluted aqueous suspensions are an important tool in medical diagnostics as contrast agent for magnetic resonance imaging and in therapy for magnetic drug targeting and hyperthermia. For these applications, special nanoparticles so called magnetosomes were isolated, which consisted of a magnetic core covered by a protein-containing lipid membrane. Under controlled synthesis conditions uniform particles of 20-45 nm core diameters may be produced which are of interest for a number of potential applications [1]. Magnetotactic bacteria are microorganisms that belong to a heterogeneous group of Gram-negative bacteria with diverse morphologies and habitats. They are a diverse group of aquatic prokaryotes [2]. Magnetotactic bacteria orient and migrate along geomagnetic field lines. This ability is based on intracellular magnetic structures, the magnetosomes, which comprise nanometer-sized, membrane-bound crystals (bacterial magnetic particles) of the magnetic iron minerals magnetite (Fe_3O_4) or greigite (Fe_3S_4) [3]. Bacterial magnetic particles can be distinguished by the regular morphology and the presence of a thin organic membrane enveloping crystals from biologically formed magnetite. Magnetosome formation is achieved by a mineralization process with biological control over the accumulation of iron and the deposition of the mineral particle with specific size and orientation within a membrane vesicle at specific locations in the cell [3].

Magnetic bacterial have an unfathomable amount of potential value for various biomedical and biotechnological applications not only scientific interests. One of potential application areas of magnetosomes is hyperthermia. The enhancement of specific heating power is of importance for reducing the useful dosage applied to the tumour. Previous investigations on the suitability of magnetic nanoparticles for hyperthermia treatments [4] and magneto-liposomes [5] have shown that biocompatible magnetite nanoparticles (with core diameter about 20 nm) are advantageous with respect to large specific heating power so the magnetosomes are of particular interest for testing their suitability for hyperthermia applications. Bacterial magnetic nanoparticles have been suggested for a number of in vitro applications such as magnetic separation and procedures for labeling and immobilization of various biomolecules. To use of magnetosomes has been described for numerous purification procedures such as the extraction of mRNA and DNA from biological samples such as tissues, blood and bacterial cells. For instance, the efficiency of DNA recovery with dendrimer-modified magnetosome particles was six fold higher with bacterial particles than with artificial magnetic particles [6]. Use of magnetotactic bacteria was given for the nondestructive domain analysis of soft magnetic materials, for locate magnetic poles on meteoritic magnetic grains, for the removal of heavy metals and radionuclides from water, for microbial magnetometer, for the immobilization of relatively large quantities of bioactive substances, which can then separated by magnetic fields, for immobilizing the enzymes glucose oxidase and uricase as components of medically important biosensors, for the generation of magnetic antibodies, for incorporated bacterial magnetite particles into eukaryotic cells, which could be manipulated by a magnetic field, for the introduction of DNA into cells, for the detection of mRNA, as a contrast agent for magnetic resonance imaging and tumor-specific drug carriers based on intratumoral enrichment, for biomedical applications [3]. As the previous attempts to characterize and apply magnetosome particles were limited by their availability, the aim of this paper is the preparation of magnetosomes and study of their structural, morphological and magnetic properties.

Materials and measuring method

Cultivation process: Bacterial magnetosomes investigated in this contribution are synthesized by magnetotactic bacteria *Magnetospirillum* sp. strain AMB-1. *Magnetospirillum* sp. strain AMB-1is a Gram-negative -proteobacterium that is more oxygen-tolerant and easier to grow on a large scale. Nowadays, the entire genome of *Magnetospirillum* sp. AMB-1 was sequenced annotated and analyzed [3]. The medium for *Magnetospirillum* sp. AMB-1 consisted of (per 1 L medium): 10 mL Wolfe's vitamin solution, 5 mL Wolfe's mineral solution, 0.68 g KH_2PO_4, 0.848 g sodium succinate hexahydrate, 0.575 g sodium tartrate dihydrate, 0.083 g sodium acetate trihydrate, 0.225 mL 0.2% (w/v) resazurin (aqueous), 0.17 g $NaNO_3$, 0.04 g ascorbic acid, 2 mL 0.01 M ferric quinate [8]. Resazurin was added to media as colorimetric indicator of redox potential. The pH was adjusted to 6.75 with NaOH. This medium was prereduced under nitrogen for a period of 1 hour, using copper as a reducing agent, and was subsequently dispensed into culture tubes in an anaerobic hood. Inoculated tubes were incubated at 25°C for a period of 4 days.

Techniques for the isolation and purification of magnetosome particles from *Magnetospirillum* species are based on magnetic separation [9,10] or a combination of a sucrose-gradient centrifugation and a magnetic separation technique [11]. These procedures leave the surrounding membrane intact and magnetosome preparations are apparently free of contaminating material. Owing to the presence of the enveloping membrane, isolated magnetosome particles form stable, well-dispersed suspensions. After solubilization of the membrane by a detergent, the remaining inorganic crystals tend to agglomerate as a result of magnetic attractive forces. Typically, 2.6 mg bacterial magnetite can be derived from a 1000-mL culture of *Magnetospirillum* sp. AMB-1. For the isolation of the magnetosome particles, we have used the method described by Gorby [9].

Isolation of magnetosomes: Approximately 10 g (wet weight) cells of *Magnetotacticum Magnetospirillum* suspended in 100 ml of 20 mM HEPES-4 mM EDTA, pH 7.4, was split up (disrupted) by sonification. The unbroken cells and the cell debris were removed from the sample by centrifugation (10 min, 3036 rpm). The cell extract was placed on to a magnet (NdFeB-magnets, 1h). The black magnetosomes sedimented at the bottom of the tube and the residual contaminating cellular material was retained in upper part tube. The residual contaminating cellular material was decanted. To eliminate the electrostatically bound contamination, the magnetic particles attached to the column were rinsed first with 50 ml of 10 mM HEPES-200 mM NaCl, pH 7.4, and subsequently with 100 ml of 10 mM HEPES, pH 7.4. After removal of the cell extract from the magnets, the magnetic particles were flushed with 10 mM HEPES buffer. The magnetosome suspension (black sedimentation) was centrifugated (18000 rpm, 4°C). After centrifugation the cell extract was placed on the magnet for 30 minutes. The magnetic particles were sedimented at the bottom of the tube, whereas residual contaminating cellular material was retained in upper part tube. The last procedure was repeated ten-times to obtain well purified magnetosomes.

Magnetization properties of prepared aqueous suspensions were measured by SQUID magnetometer of Quantum Design in magnetic field (up to 2 T) and in temperature range 2-300K. The morphological properties and size of magnetosomes were estimated from Transmission Electron Microscopy (TEM) using JEOL1200EX Microscope working at 120 kV. The samples for TEM experiments were prepared on amorphous carbon foil by micropipetted of aqueous solution of magnetosomes.

Results and discussion

It is well known that magnetosomes in magnetotactic bacteria are arranged in straight chains [1]. After isolation from these bacteria those chains tend to form closed loops. Electron micrograph of the magnetosomes (Fig.1) reveals that magnetosomes dispersed very well are arranged with tendency to form bent chains in suspension so as to minimize their magnetic stray field energy [12]. The reason for these phenomena is existence of lipid membrane surrounding magnetic core prevents them to stick together by electrostatic repulsion [3]. As was stated earlier elasticity may play a major role in the magnetosome arrangement of bent configuration [14]. The mean size and standard deviation estimated from TEM was 34 nm and 6nm, respectively. But in spite of large size distribution of magnetosomes it would be good to emphasize that in comparison to chemical preparation techniques resulting in broad lognormal distributions [13], the size distribution of magnetosomes is remarkably narrow.

Magnetization measurements of the prepared magnetosomes suspension were carried out by SQUID magnetometer of Quantum Design. The saturation magnetization of the magnetosomes was estimated to be 62 emu/g what is smaller than for chemically synthesized magnetite 75emu/g at room temperature due to presence of nonmagnetic organic layer. The curve of field dependence of magnetization at 293 K exhibited the remanence of 21 emu/g and coercivity of 185 Oe and is higher as for monodomain magnetite particles what can connected with fact that the mean diameter (34 nm) is larger than critical size for transition from superparamagnetic to ferromagnetic behaviour [12].

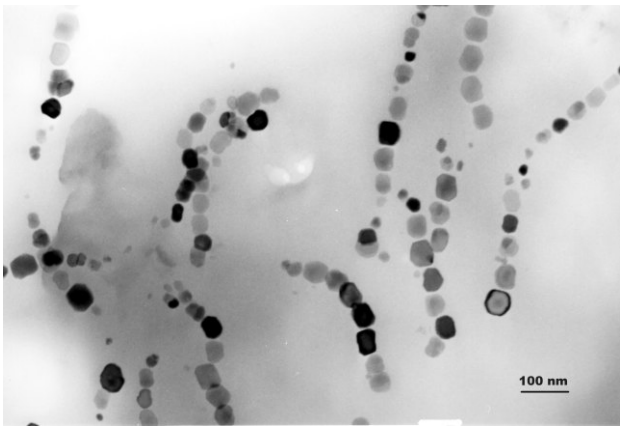

Figure 1. TEM micrograph of magnetosomes.

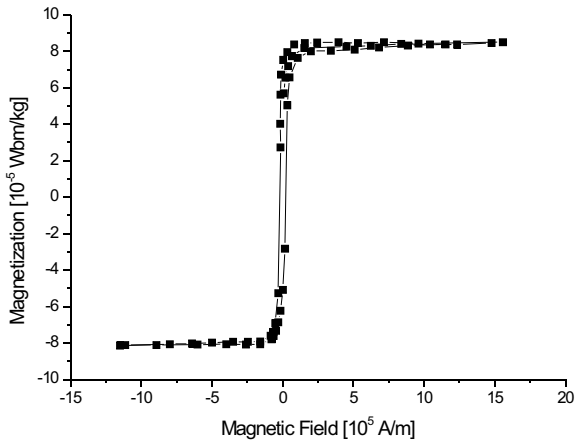

Figure 2. Magnetization of magnetosomes at 293 K.

To conclude it can be said, that formation and isolation of magnetosomes obtained by mineralization process of magnetotactic bacteria *Magnetospirillum* sp. AMB-1 was carried out succesfully. The analysis of magnetic properties has shown that magnetosomes have interesting magnetic characteristics and can replenish the chemically synthesized magnetite nanoparticles mainly in field of medical applications.

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References:

1. D. Schüler, *J. Mol. Microbiol. Biotechnol.* **1** (1999) 79.
2. T. Matsunaga and T. Sakaguchi, Molecular mechanism of magnet formation in bacteria. Department of biotechnology, Tokyo University of Agriculture and Technology Koganei. 184-8588, 2000, Japan.
3. D. Schüler, R. B. Frankel, *Appl. Microbial Biotechnol.* **52** (1999) pp.464-473.
4. R. Hergt, R. Hiergeist, M. Zeissberger, *J. Mag. Magn. Mat.* **270** (2004) 345.
5. M. Shinkai, M. Yanase, M. Suzuki, H. Honda, T. Wakabayashi, J. Yoshida, T. Kobayashi, *J. Magn. Magn. Mat.* 194 (1999) 176.
6. B. Yoza, A. Arakaki and T. Matsunaga, *J. Biotechnol.* **101** (2003) 219.
7. T. Matsunaga, Y. Okamura, Y. Fukuda, A. T. Wahyudi, Y. Murase, H. Takeyama, *DNA Research* **12** (2005) 157-166.
8. M. J. Wolin , E. A. Wolin, R. S. Wolfe, *J Biol Chem* **238**, (1963) 2882-2886.
9. Y. A. Gorby, T. J. Beverige, R. P. Blakemore: *J Bacteriol* **170** (1988)834-841.
10. Y. Okuda, K. Denda, Y. Fukumori: *Gene* **171** (1996) 99-102.
11. D. Schuler, E. Baeuerlein: The biomineralization of magnetite in magnetic bacteria. In: Trautwein A (ed) Bioinorganic chemistry: transition metals in biology and coordination chemistry. Deutsche Forschungsgemeinschaft Wiley-VCH, Weinheim New York Chichester, (1997) 24-36.
12. N. H. Hai, R. Lemoine, S. Remoboldt, *J. Magn. Magn. Mat.*, **293** (2005) 75.
13. R. Hergt, R. Hiergeist, M. Zeissberger, *J. Magn. Magn. Mat.*, **293** (2005) 80.
14. P. Valeri, J. Scherbakov, M. Winklhofer, *European Biophysics Journal* **26** (1997) 319.