BACTERIAL MAGNETITE NANOPARTICLES - MAGNETOSPIRILLUM MAGNETOTACTICUM sp. AMB-1 MAGNETOSOMES

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Abstract

The objective of this contribution is to prepare the biological magnetic nanoparticles (magnetosomes) as a product of biomineralization process of magnetotactic bacteria Magnetospirillum sp.AMB-1. at various conditions of cultivation. By adding of higher amount of Wolfe's vitamin solution (WVS) or or ferric quinate (FQ) the mean diameter of magnetosomes is increasing. Sample cultivated at normal condition (NP) shows no coercivity and behaves superparamagnetically. The increasing of coercivity (6.5 Oe for WVS and 20 Oe for FQ) may be caused by higher value of shape anisotropy and reveals stronger magnetic correlations between particles of magnetite chains. The low values for coercivity is connected with the fact that magnetosomes are still single-magnetic domain particles. The found values for the specific rate absorption (SAR) of 949 W/g for NP, 911 W/g for FQ and 890 W/g for WVS at 10 kA/m are comparable to values found for similar sample. From obtained values of SAR it can be said that only small differences exist in samples prepared various conditions.

Keywords: bacterial magnetic nanoparticles, magnetosomes, Magnetospirillum Magnetotacticum sp. AMB-1

1. INTRODUCTION

Magnetic nanoparticles offer a diverse range of applications not only in technical but also in medical applications (targeted drug delivery, hyperthermia, imaging methods, etc.). The sensitive area such as medicine can be managed successfully requires detailed preparation and characterization of magnetic nanoparticles for bio-applications. It is now handling the preparation of particles in a laboratory process called the biomineralization in magnetotactic bacteria (MTB). They create in their body the chains of magnetic crystals called magnetosomes [1].

The magnetosomes mineral phase consists of single crystals of either the ferrimagnetic iron oxide, magnetite (Fe₃O₄), or the iron sulfide, greigite (Fe₃S₄). The size of magnetosome crystals, regardless of whether they consist of magnetite or greigite, depends on the species of MTB and generally ranges from 35–120 nm. This is the size range where magnetite crystals are expected to be limited to a single magnetic domain. This means that each crystal is a tiny permanent magnet [2, 3]. Magnetic properties of MTB have been a subject of growing interest in recent years.

The advantage of these bacterial particles [4] based on iron particles is the uniform morphology and narrow size distribution and also the fact that they closed the cytoplasmic membrane (bilayer containing phospholipids and proteins) [5], preventing mutual aggregation of particles and provides biocompatibility, respectively binding of bioactive substances.

The aim of this work was isolation of magnetosomes from MTB and characterization of magnetosomes in terms of morphological, magnetically, and also hyperthermical properties with a view of bio-applications.
Techniques for the isolation and purification of magnetosome particles from Magnetospirillum sp. AMB-1 are based on combination of centrifugation and the magnetic separation [6]. Owing to the presence of the enveloping membrane, isolated magnetosome particles form stable, well-dispersed suspensions in water solution of HEPES (4-(2-hydroxyethyl)-1-piperazinethanesulfonicacid).

Magnetization measurements of the prepared magnetosomes suspension were carried out by SQUID magnetometer of Quantum Design in magnetic field up to 6 Tesla.

2. MATERIALS AND METHODS

Bacterial magnetosomes were synthesized by biomineralization process of magnetotactic bacteria Magnetospirillum strain AMB-1. The bacteria are a Gram-negative α-proteobacterium that is more oxygen-tolerant bacteria and produced magnetite - Fe₃O₄ particles. Magnetospirillum Magnetotacticum sp. AMB-1 was grown microaerobically at 25°C in grown medium for a period of 10-14 days. A microaerobic condition was establishing nitrogen, using copper as a reducing agent, and was subsequently dispersed into culture tubes for a period of 1 h. The detailed description of the cultivation process of the bacteria is given in our previous contribution [6]. In order to improve cultivation process the culture medium was changed by adding more amount FQ (ferric quinate) and more amount WVS (Wolfe’s vitamin solution) comparing to normal culture medium. For the isolation of the magnetosome particles from bacterial body, we have used the modified method described by Karen Grünberg et al. [10]. For the isolation of magnetosomes approximately 100 ml cell culture of Magnetotacticum Magnetospirillum suspended in 100 ml of 20 mM HEPES-4 mM EDTA, pH 7.4, was split up (disrupted) by signification. The unbroken cells and the cell debris were removed from the sample by centrifugation (30 min, 9000 rpm). The cell extract was placed on magnet (NdFeB-magnets, 1h). The black magnetosomes sediment 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 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 sediment) was centrifuged (18000 rpm, 30 min). After centrifugation the cell extract was placed on the magnet for 30 minutes. The magnetic particles were sediment 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.

3. RESULTS AND DISCUSSION

Typical electron micrograph of magnetosomes on surface obtained by SEM technique for prepared samples NP, FQ and WVS are shown in Figure 1a, 1b and 1c, respectively. For evaluation of different preparation conditions the size distributions of magnetosomes (from 100 particles) according to SEM photographs were prepared. The mean diameter of magnetosome prepared estimated from the size distribution of magnetosomes obtained by cultivation at normal culture (NP), more amount Wolfe’s vitamin solution (WVS) and containing more amount ferric quinate (FQ) was estimated as to be 47 nm, 52 nm and 58 nm, respectively. Very important fact was that cultivation process after adding WVS was shortening to 3-5 days and another fact that number of magnetosomes around the mean diameter was increased and distribution is narrower. Adding more quantity of FQ into the culture medium cultivation process was extended roughly 21 days. It was observed increased number of magnetosomes in part of higher and lower size of magnetosomes this causing distinct changed of size distribution and size of magnetosomes is more uniform. Increased amount solution of FQ at the cultivation medium cause averaging size of obtained magnetosomes and mean size diameter is moved to greatly higher value.
Magnetization measurements of the prepared magnetosomes suspension were carried out by SQUID magnetometer of Quantum Design. The curves of field dependence of magnetization at 293 K are reported on Figure 2 for small magnetic field to show the hysteresis of samples. These curves shows that no hysteresis loop exists at room temperature on suspension of magnetosomes prepared at normal condition (NP) and this suspension behaves superparamagnetically. Small increase of hysteresis is observed for sample WVS ($H_c = 6.5$ Oe) and for sample FQ ($H_c = 20$ Oe) what means that these samples shows ferromagnetic behavior. The reason may be caused by higher shape anisotropy, resulting in a higher coercivity for samples with higher mean diameter. The larger $H_c$ value reveals stronger magnetic correlations between particles of magnetite chains.

The heating effect of a solution with magnetosomes is a result of absorbing energy from the alternating magnetic field and converting it into heat. This phenomenon can set two ways mainly: (1) hysteresis losses during reversal of magnetization; and (2) relaxation losses accompanying demagnetization. Thermal energy from a hysteresis loss depends on the type of the remagnetization process. Over certain portions the magnetization curve is irreversible and energy of the magnetic field is dissipated into the medium with each flux-reversal cycle in the form of heat. It is known that hysteresis losses strongly depend on the size of magnetic particles. Second mechanism of the heating effect is associated with a lag between the field and magnetization due to the relaxation nature of the magnetization process in ferrofluid. There are two mechanisms by which the magnetization of a ferrofluid may relax after removing the applied magnetic field: the Brown and the Neel one. When both mechanisms act simultaneously, the mechanism with the shortest relaxation time being dominant.
Fig. 3  SAR values for the sample at $f = 508$ kHz calculated with the aid of Eq. (3) for samples NP, FQ and WVS.
In order to study heating characteristics of samples with magnetosomes the heating system consisted of sine wave power oscillator, an induction coil (solenoid with length of 78 mm and self-inductance $L_0 = 15.6 \, \mu H$ in air). Quality factor of this empty coil was $Q_0 = 121$ at frequency $f = 750$ kHz. The detailed description of measuring arrangement is given in our previous articles [7]. A glass tube containing the sample was thermally isolated by a layer of material from the solenoid winding supported on a plastic sleeve. The volume of sample equals about $0.8 \, \text{cm}^3$. The change of temperature in this time was recorded with the help of a thermocouple with accuracy of $0.01 \, \text{K}$. Hyperthermic measurements were performed at a frequency of $f = 750$ kHz vs the AC-field amplitude in the range of $0–2.5 \, \text{kA/m}$ (Figure 3). The slope of the curve $T(t)$ is a measure of the power release in a unit volume. From the fitting of the function $(\Delta T/\Delta t) = (H/a)^n$ to the experimental data the parameters $a$ and $n$ were determined which depend on several factors such as particle permeability, conductivity, size, shape and distribution. The observed $H^n$-law-type dependence of the temperature increase rate, $(\Delta T/\Delta t)_{t=0}$, on the amplitude of the magnetic field indicates the presence of superparamagnetic and partially ferromagnetic particles in the magnetic fluids studied since $n > 2$. The small amount of ferromagnetic particles causes energy losses associated with hysteresis and superparamagnetic particles cause energy losses associated with relaxation.

On the basis of the obtained relation $(\Delta T/\Delta t)_{t=0} = (H/14063)^{2.16}$ the SAR values were calculated. The SAR is defined as the amount of heat released by a unit weight of the material per unit time. It can be calculated from the expression

$$SAR_{\text{sample}} = C_S \cdot \left(\frac{\Delta T}{\Delta t}\right) \left[\frac{mW}{g_{\text{sample}}}\right],$$

where $C_S$ is the specific heat of the sample.

The results reported in [8] confirm an often-used rule that a heat deposition rate of 100 mW/cm$^3$ in tissue will suffice in most circumstances for hyperthermia therapy. The values for SAR showed that for using of magnetsome-based magnetic fluids in hyperthermia therapy the magnetic field intensities, $H_{AC}$, greater than 2.5 kA/m are needed for all samples.

The SAR data normalized with respect to the magnetite mass contents in the samples, $m_{Fe}$, can be calculated from the expression:

$$\text{SAR} = \frac{\rho C_p}{m_{Fe}} \left(\frac{\Delta T}{\Delta t}\right)_{t=0} \left[\frac{W}{g_{Fe}}\right],$$

where $\rho$ is the density of the sample and $C_p = 4.18 \, [\text{J} \cdot \text{K}^{-1} \cdot \text{g}^{-1}]$ is the sample specific heat capacity. The values of $m_{Fe}=0.003144$, $m_{Fe}=0.002341$ and $m_{Fe}=0.002954$ $[\text{g}_{Fe} \cdot \text{cm}^{-3}_{\text{sample}}]$ for sample NO, FQ and WVS, respectively, were obtained for the concentration of the magnetite grains in the ferromagnetic fluids. The found values for the SAR of 949 W/g for NP, 911 W/g for FQ and 890 W/g for WVS at 10 kA/m are comparable to values found for similar sample. From obtained values of SAR it can be said that only small differences exist in samples prepared various conditions. The existence of biocompatible phospholipidic membrane around magnetosomes and the obtained SAR values show that magnetosomes may be considered as good materials for the biomedical applications in hyperthermia.
4. CONCLUSION

From obtained results it can be concluded that the chains of magnetite behaves superparamagnetically for sample cultivated at normal condition and display partly ferromagnetic properties at room temperature for sample WVS and FQ. The increasing of coercivity for sample WVS and FQ is connected with higher shape anisotropy for samples with higher mean size diameter. As specific steps involved in magnetosome biomineralization process are still under dispute, our contribution showed that the changes of cultivation conditions can change the duration of process, morphology of magnetosome and consecutively the magnetic properties. So this type research can offer some useful information to understand the biomineralization process. From hyperthermia experiment it can be seen that condition of sample preparation changes the specific absorption rate. As coercive force is increasing for sample FQ and WVS it would be normally to obtain higher values for SAR but our experiment show that SAR value for sample FQ and WVS are smaller as for sample NP prepared at normal condition. It seems that the main reason for heating process is the rotation of all chains and not only relaxation or hysteresis processes in consequence of large value of magnetocrystalline anisotropy due to magnetosome arranged in chains.

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LITERATURE


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