Magnetic properties and application of biomineral particles produced by bacterial culture

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Abstract

Ferrihydrite nanoparticles (2–5 nm in size) produced by bacteria culture in the course of biomineralization of iron salt solutions from a natural medium exhibit unique magnetic properties: they are characterized by both the antiferromagnetic order inherent in a bulk ferrihydrite and the spontaneous magnetic moment due to the decompensation of spins in sublattices of a nanoparticle. The magnetic susceptibility enhanced by the superantiferromagnetism effect and the magnetic moment independent of the magnetic field provide the possibility of magnetically controlling these natural objects. This has opened up the possibilities for their use in nanomedicine and bioengineering. The results obtained from measurements of the magnetic properties of the bacterial ferrihydrite in its two main crystalline modifications are reported. This has made it possible to determine numerical values of the magnetic parameters of real biomineral nanoparticles.

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1. Introduction

Nanoparticles have attracted interest of scientists in various areas of biomedical research because of the unique properties of the nanomaterials. It is already known that one of the major breakthroughs in the relative application of nanoparticles is the realization of the steric stabilization which can increase the particle stability in the biological environment and provide the opportunities of the application of nanoparticles in drug delivery systems for achieving drug targeting and controlled drug release.

Nanoscale ferrimagnetic particles have a diverse range of uses from directed cancer therapy and drug delivery systems to magnetic recording media and transducers. Such applications require the production of monodisperse nanoparticles with well-controlled size, composition, and magnetic properties. To fabricate these materials purely using synthetic methods is costly in both environmental and economical terms. However, metal-reducing microorganisms offer an untapped resource to produce these materials [1].

By all appearances, unlike magnetosensitive bacteria Magnetospirillum, the magnetization of biomineral nanoparticles is of little significance for our bacterial culture. Most likely, that is why the magnetic properties of the ferrihydrite of these bacteria have not been adequately investigated.

However, it is known from the extensive literature devoted to the ferritins of humans and animals [2-5] that these properties are well measurable and sufficiently pronounced that the ferrihydrite can be used in magnetic nanomedicine and bioengineering.

In this work, we study the ferrihydrite nanoparticles (2–5 nm in size) produced by bacteria in the course of biomineralization of iron salt solutions from a natural medium an exhibit unique magnetic properties. Since these bacteria are easily reproduced under laboratory conditions, they can be used as "biofactories" for manufacturing these nanoparticles. As was shown in our earlier works [5-8], bacterial culture during the growth produces the ferrihydrite of two types. The differences between these modifications are well identified, and their quantitative ratio varies with time in a nonmonotonic manner. This inference was made for the first time from analyzing the Mössbauer spectra [8]. Below, we will demonstrate that both types of nanoparticles can be identified using a more simple technique, i.e., static magnetic measurements.

2. Materials and methods

The strain Klebsiella oxytoca was used for producing of the biomineral ferrihydrite nanoparticles. Biomass was separated from the sapropel of Lake Borovoe (Krasnoyarsk region, Russia) by passing the taken samples through a magnetic separator. A bacterial biomass was grown under the microaerophilic and aerophilic conditions on a Lovley medium of the following composition: NaHCO₃, 2.5 g/l; CaCl₂ · H₂O, 0.1 g/l; KCl, 0.1 g/l; NH₄Cl, 1.5 g/l; and NaH₂PO₄ · H₂O, 0.6 g/l. The ferric citrate concentration was equal to 0.5 g/l, and the yeast extract concentration was 0.05 g/l. Ferrihydrite nanoparticles were extracted from bacterial culture by polygradient separation method and biochemical methods for creating stable colloidal solution in a medium.

In Mössbauer measurements, we used a 57Co(Cr) gamma source with a full width at half maximum of 0.24 mm/s for a sodium nitroprusside powder absorber. The sample thickness was 5–10 mg/cm² in terms of iron content, which ensured a linear relation between absorption and the Fe content of the phase of interest.

The biomass was divided in two factions (magnetic and nonmagnetic) by magnetic polygradient separation. In the magnetic polygradient separation was taken electromagnet PL. Power source of electromagnet generated electric current of 10A, that corresponded to value field in the electromagnet gap 12.6 kOe. The biomass equal amounts were passed through magnets. Non-magnetic fraction had been accumulated in the receiver and the magnetic fraction remained in the magnet. Magnet fraction was washed from samples, then both products were settled and the water was decanted.

Cytotoxicity of magnetic nanoparticles was studied by chemiluminescence analysis carried out on neutrophils of healthy people exposed with magnetic nanoparticles in vitro.

3. Results and discussion

According to the Mössbauer spectroscopic investigations, the biomineral nanoparticles contain two magnetically ordered phases. Each phase is characterized by two states of Fe³⁺ ions with close values of the quadrupole splitting. In the phase conventionally termed Fe(12), the quadrupole splitting is equal to 0.6–1.0 nm/s. In the phase called Fe(34), the quadrupole splitting lies in the range 1.5–1.8 nm/s.
The main difference lies in these phases in the magnetic susceptibility. The parameter \( \chi_m \) for the Fe(12) particle is close to \( 10^{-4} \) cm\(^3\)/g, which is approximately three times larger than that for the Fe(34) phase [9]. In the Mössbauer spectra, the quadrupole splitting serves as the comparison parameter. According to the quadrupole splitting, the inference was made that the Fe(12) crystallites are more ordered as compared to the Fe(34) crystallites.

The magnetic fraction of polygradient separation products is mainly represented by phase Fe(12), and nonmagnetic phase Fe(34), it also shows the differences of magnetic susceptibilities of phases Fe(12) and Fe(34). The results of Mössbauer spectroscopy are represented on the Fig. 1.

Fig. 1. Mössbauer spectra of bacteria and the corresponding probability distribution functions of quadrupole splitting by products of separation.

The samples were also studied by small angle X-ray (SAXS) method on Brucker Nanostar instrument available at the Institute of Synthetic Polymer Materials RAS, Moscow. The experimental setup used covers the \( Q \) range of \( 0.01 \) – \( 0.11 \) Å\(^{-1}\). In Fig. 2 experimental and fitting curves are presented.

Fig. 2 Small angle X-ray scattering experimental curves from samples Fe12 and Fe34 obtained at Brucker Nanostar SAXS spectrometer at the Institute of Synthetic Polymer Materials RAS, Moscow [10].
4. Conclusions

Thus, it has been demonstrated that the magnetic measurements of nanoparticles consisting of the bacterial ferrihydrite make it possible to identify the presence of two different modifications of this compound, i.e., the Fe(12) and Fe(34) phases, in the samples. As a result, we have established that the structural differences revealed between crystallites of these modifications with the use of Mössbauer spectroscopy correlate well with the differences in their magnetic properties.

According to the magnetic grain-size estimates, the sizes of crystallites of both types are close to each other (1.5–2.0 nm); however, the magnetic susceptibilities of the Fe(12) and Fe(34) particles differ substantially.

The established relationships between the magnetic and structural properties of the Fe(12) and Fe(34) phases significantly enhance the instrumental capabilities of investigating the bacterial ferrihydrite. Collected information on the magnetism of biominal particles is useful for developing applications that suggest the use of natural antiferromagnetic nanodispersions as magnetically controlled functional materials.

Revealed that the magnetic nanoparticles do not affect the activity of neutrophils, indicating, that the particles haven’t cytotoxicity. The absent of toxic effects of nanoparticles provided the basis for studying the effectiveness of magnetic nanoparticles in combination with antibacterial drugs for their controlled delivery to the affected tissues. We demonstrated the presence of weak antitumor activity of drugs, which absorbed on iron particles, against Ehrlich ascites carcinoma in mice. However, the biological properties of these substances require further studies.

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Reference