Synthesis and Characterization of Magnetoferritin

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The paper presents detailed experimental study of synthesis and characterization a bioinorganic magnetic molecule — magnetoferritin. Magnetoferritin with loading of iron ions per protein molecule in the range from 300 to 3000 was prepared. Size distribution analysis (transmission electron microscopy, dynamic light scattering) shows spherical nanoparticles with particle size distribution from 2 to 12 nm, and hydrodynamic diameter from 12 to 25 nm. The thermomagnetic curves measured after cooling the sample in zero field (zero-field cooling) and under the presence of the measurement field (field cooling) show superparamagnetic behavior with the blocking temperature $T_b$ from 22 to 60 K and the magnetization loops measured below $T_b$ (at 2 K) show the hysteresis with coercive field from 20 to 30 kA/m depending on the concentration of the magnetic nanoparticles.

PACS: 75.30.Cr, 75.50.Ee, 75.60.Ej, 75.50.Tt

1. Introduction

In 1992, it was shown that the cavity of apoferritin, the empty form of the protein, can be used as a confined reaction vessel to synthesize nanoparticles of non-native compounds [1]. The first of these was maghemite, a ferrimagnetic iron oxide ($\gamma$-Fe$_2$O$_3$). The resulting material has been, for this reason, named "magnetoferritin" [1–3]. Magnetoferritin is a protein capsule (apoferritin) with a structure close to spherical layer (inner and outer diameter of 8 and 12 nm, respectively) filled with magnetic nanoparticles. Along with it, magnetoferritin proved to be a useful model system for studying the fundamental effects of magnetostatic interactions in nanoparticle assemblies. Magnetic nanoparticles grown in these biological moulds are usually rather homogeneous in size, free from aggregation and soluble in water. Other important advantages, especially for applications, are their biocompatible character (magnetic concentrating of anticancer drugs in tumors, magnetic resonance imaging, magnetic hyperthermia cell labeling, etc. [4–7]), and the possibility to process them in order to fabricate complex superstructures [8] and even ordered crystals [9, 10].

The paper presents detailed experimental study of synthesis and characterization a bioinorganic magnetic molecule — magnetoferritin.

2. Experimental

Synthetic ferritin, i.e., magnetoferritin was synthesized from equine spleen apoferritin (Sigma-Aldrich). Aqueous solutions of Fe$_3$O(NO$_3$)$_2$ (0.07 M) and 0.05 M buffer solution AMPSO (3-(1,1-dimethyl-2-hydroxyethyl)aminol-2-hydroxypropanesulfonic acid) buffered to pH 8.6 with 2 M NaOH were prepared. The buffer was degassed for 30 min with nitrogen. Then 1.5 μM solution of apoferritin (AF) in AMPSO was prepared and continued with deaeration for a further 30 min then it was hermetically closed and placed in a preheated (65 °C) water bath on magnetic stirrer until the contents were allowed to reach equilibrium. For the synthesis of magnetoferritin 0.1 M ferrous ammonium sulfate was prepared with deaerated water. Gentle stirring was continued and aliquots of Fe(II) and Fe$_3$O(NO$_3$)$_2$ were added dropwise to the reaction solution using syringes. In general, each addition of Fe(II) was followed by a stoichiometric aliquot of Fe$_3$O(NO$_3$)$_2$ (3Fe(II) : 2Fe$_3$O(NO$_3$)$_2$) and the solution left for 15 min before repeating the stepwise procedure. Finally it was dialyzed against distilled water for 24 h to remove free ions from reaction solution.

Product was determined spectrophotometrically (UV-Vis spectrophotometer SPECORD 40, Analytik Jena). The amount of iron was measured after HCl/H$_2$O$_2$ induced oxidation of Fe(II) to Fe(III) and an addition of 1% ammonium thiocyanate by absorption measurement of the thiocyanate complex at $\lambda = 450$ nm. The content of protein was detected by modified Bradford method at $\lambda = 595$ nm.

The morphology and size distribution of the samples were determined with the transmission electron microscopy (TEM Tesla BS 500). The sample was dropped on a copper grid and dried in air. The hydrodynamic size was determined by Zetasizer Nano system from Malvern Instruments. Here, the fluctuations in the scattered light are analyzed to detect the diffusion of the molecules and deduce their hydrodynamic size. The magnetic properties of the samples were performed with a SQUID magnetometer (Quantum Design MPMS 5XL) up to 5 T in the temperature range 2–295 K. For all temperatures and fields, there were measured both the signal of the solution containing magnetoferritin (samples B–G) and the signal of the solution containing apoferritin (sample A — the empty protein shells) with the same protein concentration. After subtraction of the second signal from the first, we thus obtain magnetization values due only to the magnetoferritin cores.

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3. Results and discussion

The sphere that is formed by apoferitin is approximately 12 nm in diameter (Table). This is a rather large protein, yet despite its unusual structure it follows the size predictions for globular proteins quite well. The number of Fe ions amount per molecule (number $N$), hydrodynamic size and magnetic properties are summarized in the Table.

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Hydrodynamic diameter is an effective diameter of the protein in water which includes all the water molecules attracted to the molecule. It depends not only on the size of the mineral core of magnetoferritin, but also on surface of the molecule. Hydrodynamic diameter is growing with the increase of number $N$. The sample with highest Fe loading (sample G) shows aggregation of molecules due to magnetic force between nanoparticles in protein shell. Sample H in Table represents native horse spleen ferritin. It is applied because of comparison with synthesized magnetoferritin. Ferritin is by the shape like magnetoferritin, but its magnetic core, which is structurally similar to the mineral ferrhypyrrite ($\delta$Fe$_2$O$_3$·9H$_2$O), presumes different magnetic properties. The TEM analyzes only magnetic nanoparticles encased in the protein shell. It shows increase of magnetic nanoparticles with the increase of number $N$ in the range from 2 nm to 12 nm. These results are in good agreement with the measured hydrodynamic size.

The magnetic measurements show superparamagnetism of prepared magnetic particles without hysteresis at room temperature. The thermomagnetic curves (Fig. 1) measured after cooling the sample in zero field (ZFC) and under the presence of the measurement field (FC) show superparamagnetic behavior with the blocking temperature $T_b$ around 25 K for samples with the low number $N$ (samples B, C, D, E). For higher number $N$ the blocking temperature is smeared due to aggregation of the particles. The magnetization loops measured below $T_b$ (at 2 K, Fig. 2) show the hysteresis with coercive field from 20 to 30 kA/m depending on the iron loading. The magnetization undergoes a slow approach to saturation but it is not yet saturated at fields up to 5 T which can be achieved. This result needs a further investigation.

The value of magnetization increases with increase of number $N$ as it is shown in Table. The high field magnetization measurements of related sample, native horse spleen ferritin, are published in [12]. The magnetic birefringence study of magnetoferritin is published in [13].

Fig. 1. The ZFC–FC curves measured at 10 mT.
4. Conclusions

We have prepared and characterized magnetoferritin with various iron loading per protein molecule. Size distribution analysis (TEM, dynamic light scattering (DLS)) confirmed spherical nanoparticles with particle size distribution from 2 to 12 nm, and hydrodynamic diameter from 11 to 25 nm. The magnetic measurements have showed superparamagnetism of prepared magnetic particles without hysteresis at room temperature and the hysteresis with coercive field from 20 to 30 kA/m depending on the concentration of the magnetic nanoparticles below \( T_b \) (at 2 K). The thermomagnetic curves measured after cooling the sample in zero field (ZFC) and under the presence of the magnetic field (FC) show superparamagnetic behavior with the blocking temperature \( T_b \) around 25 K for samples with the lower loading of Fe. For higher loading of Fe the blocking temperature is smeared due to aggregation of the particles.

Acknowledgments

This work was supported by the Slovak Research and Development Agency under the contract No. APVV-0171-10, by the projects Nos. 2620120021, 262201200033 in the frame of Structural Funds of European Union, Centre of Excellence of SAS Nanofilm and VEGA 0077.

References