MAGNETOFERRITIN

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Abstract

Magnetoferritin is a biomacromolecule, which consists of a spherical protein shell of external diameter 12 nm, with an inner cavity of approximately 8 nm diameter. Iron is stored within this cavity as particles of the Fe3O4, Fe2O3. Magnetoferritin may have considerable importance as a biocompatible ferrofluid, with many possible biomedical and industrial applications based on its magnetic properties. Here we present detailed experimental study of synthesis and characterization of morphological and magnetic properties of magnetoferritin with various iron loading per protein molecule in the range from 300 to 3000. Synthesis was made by addition of increments of Fe(II) to anaerobic solutions of apoferritin, at pH = 8.6 and 65 °C, followed by stoichiometric amounts of the oxidant. Product was determined spectrophotometrically. The amount of iron was measured at λ = 450 nm and the content of protein was detected by modified Bradford method at λ = 595 nm. The structural characterization of the inorganic cores was determined by TEM and X-ray diffraction. Hydrodynamic diameter of the nanoparticles was determined by non-invasive back scatter technology by Zeta sizer. The results obtained from TEM and Zeta seizer shows increasing the diameter with the iron loading. Magnetic properties were investigated by a SQUID magnetometer. The magnetic measurements show superparamagnetism of prepared magnetic particles without hysteresis at room temperature.

Keywords: Ferritin, Magnetoferritin, Magnetic nanoparticles, Superparamagnetism

1. INTRODUCTION

While the core of naturally occurring ferritin is comprised of the hydrous iron oxide ferrihydrite, the ferrimagnetic core of magnetoferritin is comprised of the iron-oxide ferrite magnetite, Fe3O4, or maghemite, γ-Fe2O3, or an intermediate composition. Ferritin, a protein naturally occurring in bacteria, fungi, plants, invertebrates and vertebrates including humans, is characterized by an ability to sequester and store iron in a bioavailable form [1, 2]. Chemically, the protein is a quaternary structure of 24 polypeptide units assembled into a spherical shell having an internal cavity about 8-10 nm. In naturally occurring ferritin, the cavity contains up to 4500 iron atoms as a mineral core of the hydrous iron oxide ferrihydrite which is paramagnetic at room temperature. 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 [3]. The first of these was maghemite, a ferrimagnetic iron oxide (γ-Fe2O3). The resulting material has been, for this reason, named ‘magnetoferritin’ [3–5]. 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 [6] and the possibility to process them in order to fabricate complex superstructures [6] and even ordered crystals [7, 8].

The paper presents detailed experimental study of synthesis and characterization a bioinorganic magnetic molecule – magnetoferritin
2. MATERIALS AND METHODS

Synthetic ferritin, i.e., magnetoferritin was derived from equine spleen apoferritin (Sigma – Aldrich) using the synthesis scheme (fig.1). Aqueous solutions of Me$_3$NO (0.07 M) and 0.05 M buffer solution AMPSO (3-[(1,1-Dimethyl-2-hydroxyethyl)amino]-2-hydroxy-propanesulfonic acid) buffered to pH 8.6 with 2 M NaOH were prepared. The buffer was deaerated 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 enclosed 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 Me$_3$NO were added dropwise to the reaction solution using syringes. In general, each addition of Fe(II) was followed by a stoichiometric aliquot of Me$_3$NO (3Fe(II):2Me$_3$NO) and the solution left for 15 min before repeating the stepwise procedure. Samples with different theoretical loadings from 300 to 3000, Fe atoms/protein molecule were prepared. Finally it was dialyzed against distilled water for 24 hours to remove free ions from reaction solution.

Product was determined spectrophotometrically (UV-Vis spectrophotometer SPECORD 40, Analytik Jena). It was determined the loading of iron amount per molecule (number N). 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 λ = 450 nm. The content of protein was detected by modified Bradford method at λ 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 on the air. The hydrodynamic diameter was studied by dynamic light scattering method and the isoelectric point using Laser Doppler velocity by the measuring Zeta potential on Zetasizer nano Malvern Instrument. Here, the fluctuations in the scattered light are analyzed to detect the diffusion of the molecules and deduct their hydrodynamic size. The magnetic properties of the samples were performed with a SQUID magnetometer (Quantum Design MPMS 5XL) up to 6 T in the temperature range 2.0 - 295 K. For all temperatures and fields, 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.

3. RESULTS AND DISCUSSION

3.1 Analytical methods

The figure 2 indicates a highly monodisperse sample of apoferritin and magnetoferritin with only one oligomeric species present in solution. For the magnetoferritin samples are evidence of a broad distribution. The distribution of the hydrodynamic diameter increases by the number N.
The sphere that is formed by apoferritin is approximately 12 nm in diameter (Tab. 1). This is a rather large protein, yet despite its unusual structure it follows the size predictions for globular proteins quite well. The hydrodynamic size is growing with the increasing of number $N$. The sample with higher Fe loading (Sample G) shows aggregation of molecules due to magnetic force between nanoparticles in protein shell. Sample H in the table 1 represents native horse spleen ferritin. It is applied because of comparison with synthesized magnetoferritin.

Table 1 Summary of analytical measurements

<table>
<thead>
<tr>
<th>Sample</th>
<th>(A)</th>
<th>(B)</th>
<th>(C)</th>
<th>(D)</th>
<th>(E)</th>
<th>(F)</th>
<th>(G)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number $N$</td>
<td>0</td>
<td>320</td>
<td>485</td>
<td>540</td>
<td>1250</td>
<td>1937</td>
<td>3023</td>
</tr>
<tr>
<td>Hydrodynamic diameter (nm)</td>
<td>11.76</td>
<td>13.95</td>
<td>13.16</td>
<td>18.40</td>
<td>16.25</td>
<td>24.13</td>
<td>655; 4600 (agglomer)</td>
</tr>
</tbody>
</table>

TEM examination of the samples revealed crystals of different size. Fig 3 shows increasing of the spherical magnetic nanoparticles encased in the protein shell with the increasing of number $N$ in the range from 2 nm to 12 nm. The black spots correspond to the iron oxide cores.

The size distribution histogram shows, that in the sample (B) are 18% particles with the diameter 2 nm, 48% with 4 nm and 24% with diameter 6 nm. In the sample (E) it is following: 4 nm – 48%, 5 nm – 58%, 6 nm – 14%. The figure 2 shows, that the particles of the sample (F) are clustered after drying on the copper grid.
Electron diffraction patterns were measured on selected areas of all samples. Illustrative examples are shown in figure 4. Electron diffraction identified the crystals formed in the reactions to be crystalline magnetite. This conclusion was confirmed by X-Ray Diffraction analysis.

### 3.2 The stability evaluation

The isoelectric point (IEP) is defined as the point of zero zeta potential. For a sample which is electrostatically stabilized, the IEP is often the point of least stability due to the repulsive forces being weakest. This may be important when considering the shelf life of a product, as normally the sample needs to be away from the IEP. Figure 5 shows, as illustrative example, the plot of the zeta potential of the sample (E) measured as a function of pH. As titration reagent was used 0.01 M HCl. The isoelectric point of the samples is at pH 4.52, it is consistent with the results published by S. T. Silk and E. Breslow [9], who get the IEP for ferritin and apoferritin equal 4.58 ± 0.02. In addition, the plot can be used to predict that the sample should be stable at the extremes of pH.

![Fig. 4 The electron diffraction of the samples (B) and (E).](image)

### 3.3 Evaluation of magnetic properties

The magnetic measurements show superparamagnetism of prepared magnetic particles without hysteresis at room temperature. The thermomagnetic curves (Fig. 6) measured after cooling the sample in zero field (ZFC) and under the presence of the measurement field (FC) show superparamagnetic blocking 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 $T_b$ (Fig. 1) show the hysteresis with coercive field from 16 to 36 kA/m depend on the iron loading. The magnetization undergoes a slow approach to saturation. Clearly, magnetization at 6 T is not yet saturated at fields which we can achieve. This result needs a further investigation.

![Fig. 5 A plot of the zeta potential of the sample (E) measured as a function of pH](image)
CONCLUSIONS

We prepared and characterized magnetoferritin with various iron loading per protein molecule. Size distribution analysis (TEM, DLS) shows spherical nanoparticles with particle size distribution from 2 to 12 nm, and hydrodynamic diameter from 11 to 25 nm. The determination of isoelectric points is important in understanding the stability of colloidal dispersions. Magnetoferritin was characterized by the combination of three light scattering techniques. The combination of the information of the size, and the charge may be employed to control and ultimately produce better nanotechnology products in the future. In addition, its biological compatibility may allow its use for biomedical applications. The magnetic measurements show superparamagnetism of prepared magnetic particles without hysteresis at room temperature and the hysteresis with coercive field from 16 to 36 kA/m depend 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 measurement 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.

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LITERATURE

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