Small-Angle Scattering on Magnetoferritin Nanoparticles

L Balejčíková¹, VI Petrenko²,³, MV Avdeev², VM Garamus⁴, L Almásy⁵,⁶ and P Kopčanský¹

¹Institute of Experimental Physics Slovak Academy of Sciences, Watsonova 47, 04001 Košice
²Joint Institute for Nuclear Research, Joliot-Curie 6, 141980 Dubna, Moscow region, Russia
³Taras Shevchenko National University of Kyiv, Volodymyrska Street 64, Kyiv, 01033 Ukraine
⁴Helmholtz-Zentrum Geesthacht: Centre for Materials and Coastal Research, Max-Planck-Street 1, 21502 Geesthacht, Germany
⁵State Key Laboratory Cultivation Base for Nonmetal Composites and Functional Materials, Southwest University of Science and Technology, Mianyang 621010, China
⁶Wigner Research Centre for Physics, Hungarian Academy of Sciences, H-1525 Budapest, POB 49, Hungary

balejcikova@saske.sk

Abstract. Magnetoferritin is a synthetically prepared magnetic bio-complex, consisting of apoferritin shell and iron-based nanoparticles. Superparamagnetic behaviour, nanoscale size (about 12 nm) and biological origin allow to use magnetoferritin in various applications. In this report, we present a general overview about basic physicochemical properties of magnetoferritin, as determined by small-angle X-ray and neutron scattering experiments and some interesting references on their potential bio-applications.

1. Introduction

Iron-based magnetic nanoparticles are interesting in wide range of applications. In liquid dispersions, they tend to agglomerate due to attractive magnetic interactions. To secure their colloidal stability and homogeneity in aqueous or oily solutions, magnetic nanoparticles should be coated by various organic polymers. Such prepared magnetic fluids are used especially in biomedicine, nanotechnology and industry. For minimization of their toxicity and increasing of biocompatibility in possible biomedical applications, magnetic nanoparticles can be bound also through DNA (e.g. plasmids), phospholipids, or proteins [1]. In 1991 it was shown that the empty cavity of the iron-storage protein ferritin, entitled apoferitin, could be used as a reaction medium for in vitro chemical synthesis of iron-based nanoparticles [2]. The first synthesis of such material, called magnetoferritin, was published in 1992 [3]. Since then, many physicochemical properties have been examined and its synthesis technology is continuously improving. In the recent years, we carried out systematic investigations on magnetoferritin prepared at various synthesis conditions and at various loading factors (i.e. the average number of iron atoms per one apoferitin). The overview of basic physicochemical properties investigated using available techniques, significant results obtained by small-angle X-ray (SAXS) and neutron (SANS) scattering, and potential bio-applications of magnetoferritin are described in this paper. Numerous experimental methods were used in our studies in order to better understand physico-
chemical properties of magnetoferritin. Synthesis technology modification allow to obtain magnetoferritin samples with different loading factors, colloidal stability, size distribution and magnetisation, demanding for thorough characterization of magnetoferritin using advanced physico-chemical methods. For the first time we investigated magneto-optical and structural properties of magnetoferritin colloidal solutions using structure-sensitive methods such as Cotton-Mouton and Faraday rotation measurements and SAXS/SANS. The obtained results had significant contribution for better understanding the magnetoferritin behavior, and first of all, its structure, under well defined physico-chemical conditions. Characterization of the structural changes of magnetoferritin at various conditions is indispensable for understanding its behavior and interactions with biological media in the perspective biomedical applications.

2. Basic physicochemical properties of magnetoferritin
Magnetoferritin aqueous colloidal solution prepared by controlled chemical synthesis with a procedure described previously [4] was characterized by zeta (ζ) potential measurements, dynamic light scattering (DLS), transmission electron microscopy (TEM) and SQUID magnetometry. The ζ-potentials of the apoferritin and magnetoferritin at comparable concentrations were −25.5 and −21.9 mV, respectively. These measurements confirmed negative surface charge of the protein and good colloidal stability. The hydrodynamic size of magnetoferritin was investigated by dynamic light scattering technique and compared with apoferritin. The average hydrodynamic diameter for magnetoferritin increases (⟨DHYDR⟩ = 19.5 nm) in comparison with pure apoferritin hollow sphere (⟨DHYDR⟩ = 14.1 nm). This increase of hydrated/solvated protein macromolecules can be related to partial destruction and agglomeration of a small fraction of the proteins upon loading iron. The majority of the iron oxide phase is still inside the inner cavity of apoferritin while the presence of nanocrystallites with an average diameter of 5 nm have been revealed by TEM [5],[6].

Magnetic properties of magnetoferritin were studied using a SQUID magnetometer in magnetic fields up to 4000 kA/m. Magnetic-field dependencies have shown superparamagnetic behaviour of system without hysteresis at room temperature. Hysteresis was observed below a blocking temperature (ȠB = 26 K) at 2 K with a coercive field of 20 kA/m [4] -[6].

Sensitive and non-invasive magneto-optical methods allowed to distinguish between magnetoferritin and ferritin. Magneto-optical Cotton-Mouton effect is generated thanks to the optical anisotropy of magnetic nanoparticles and orientation of their magnetic moments in an applied magnetic field. The electromagnetic radiation which pass through the sample causes rotation of the polarization plane of light and change the angle, which is proportional to the birefringence. Direct measurements of magnetically induced optical linear birefringence normalised to iron concentration as a function of the applied magnetic field intensity square H² at the wavelength 632.8 nm and 295 K indicated differences in magneto-optical behaviour of aqueous dispersion of magnetoferritin in comparison with pure ferritin solution. Cotton-Mouton constant for magnetoferritin, calculated at low region of magnetic field below 200 Oe was about 4 orders of magnitude higher than for ferritin that was related to the presence of iron containing core inside both biomacromolecules. This magneto-optical study allows identification of magnetic core of the unknown sample of ferritin and its biogenic or synthetic derivatives [7], [8].

The Faraday magneto-optical effect, similar as the Cotton-Mouton effect, is based on the rotation of the polarization plane of light depending on the intensity of the applied magnetic field. Faraday rotation spectra for magnetoferritin in comparison with commercial ferritin in the applied magnetic field with the intensity of H = 2970 Oe in the spectral range of the used light with the wavelength from 300 to 680 nm have shown significant differences, which are related to the various phase composition of iron cores. The dependence of Faraday rotation on magnetic field has shown superparamagnetic behaviour of magnetoferritin [9], what is in good agreement with our previous investigations [4] -[5]. The calculated Verdet constant could, after standardization, serve as a suitable parameter for quantitative determination of the iron oxidation state of various nanoparticles with a specific size. Cotton-Mouton and Faraday magneto-optical effects could be useful in biomedical applications for the detection of iron oxides in pathological tissues (e.g. in diagnosis of neurodegenerative diseases) [9].
3. Small-angle scattering on magnetoferritin nanoparticles

Small-angle scattering of X-rays (SAXS) and neutrons (SANS) was applied for the structural study of magnetoferritin nanoparticles in colloidal solution. The first experiments, performed with full loading of iron, have shown large difference between scattering curves of magnetoferritin in comparison with pure apoferritin solution \cite{5,6}, as a result of the presence of the new component. Therefore, further investigations were focused on the behaviour with relatively low iron content (i.e. loading factor, the average iron atoms per one apoferritin) expecting only a weak change in the protein structure. Nevertheless, scattering curves of magnetoferritin with loading factor 160 in comparison with pure apoferritin were markedly different (Fig. 1). \textit{Ab initio} analysis points to partial destruction of the magnetoferritin shell (Fig. 1). It should be noted that the structure obtained in the modelling represents the average shape and does not exclude the existence of complete shells.

For better understanding the effect of iron loading on the magnetoferritin structure, SAXS measurements were performed for different loading factors. Here, the scattering curves and the calculated pair distance distribution functions have shown an increase of aggregation with the loading factor growth. Comparisons of radius of gyration and hydrodynamic diameter obtained by dynamic light scattering, have shown that the magnetoferritin particles size increases with the loading factor and a strong aggregation occurs at the loading factor over 600 \cite{10}.

![Figure 1. SAXS scattering curves for apoferritin and magnetoferritin at the loading factor 160 with the corresponding \textit{ab initio} models. Figure taken from \cite{10} with permission.](image-url)

Next investigation was SANS contrast variation study of magnetoferritin with different loading factors. The difference between the neutron scattering length density for light ($\rho_{\text{H}_2\text{O}} = -0.559 \times 10^{10}$ cm$^{-2}$) and heavy water ($\rho_{\text{D}_2\text{O}} = 6.34 \times 10^{10}$ cm$^{-2}$) can be used for the variation of contrast by varying the light and heavy water ratio in the solution. Forward scattering intensities measured at the different heavy water content allowed to obtain match points at 0.434, 0.484 and 0.495 volume fractions of D$_2$O, for loading factors of 160, 510 and 770, respectively. Assuming homogeneous and monodisperse approximation of the magnetoferritin core containing magnetite, the calculated scattering length densities were obtained as $2.46 \times 10^{10}$ cm$^{-2}$, $2.79 \times 10^{10}$ cm$^{-2}$ and $2.86 \times 10^{10}$ cm$^{-2}$, for loading factors 160, 510 and 770, respectively, in comparison with SLD of apoferritin $2.34 \times 10^{10}$ cm$^{-2}$. Volume fractions of magnetic part in magnetoferritin were 0.026, 0.099 and 0.114 for loading factor 160, 510 and 770, assuming magnetite or maghemite form of the iron oxide phase. SANS contrast variation
indicated an abnormally high ratio of the amount of the magnetic component. The reason could be associated with partial shell destruction, observed also from SAXS data. On the other hand, the non-zero intensity in the match point indicated the size and structural polydispersity growth with increasing of loading factor, what could be explained also by the presence of unknown iron phases in magnetoferritin [10],[11]. Protein shell destroying could be caused by the specific effect of the iron oxide nanoparticles.

Many studies confirmed that various nanoparticles with specific physicochemical properties affect proteins in different way. Understanding of these interactions is the main subject of intensive research in recent years. Explanation of this phenomenon requires further detailed studies, aimed to optimize the synthesis technology and use of suitable separation techniques for future bio-applications of magnetic nanoparticles complexes with proteins.

In the latest SAXS study the effect of magnetoferritin on the structure of lysozyme amyloid aggregates was investigated. It was observed that magnetoferritin is able destroy the lysozyme amyloid fibrils. The lysozyme amyloid fibrils size reduction, confirmed by the pair-distance distribution function analysis (Fig. 2) was supported by the fluorescence spectrophotometry measurements as the decreasing of lysozyme amyloid fibrils amount after incubation with magnetoferritin. Magnetoferritin and lysozyme amyloid fibrils due to their biological origin are suitable pathological model systems for better understanding neurodegenerative diseases mechanism, and the presence of magnetic nanoparticles in magnetoferritin seems to play an important role in the amyloid destruction [12]. Moreover, magnetoferritin can be useful for potential applications as therapeutic agent against neurodegenerative diseases development. The precise mechanism of interaction of magnetic nanoparticles with lysozyme amyloid fibrils was not established yet. Such interactions could depend on nanoparticles phase composition, concentration, surface, charge distribution, physicochemical condition of surrounding medium (e.g. pH, temperature, ionic strength).

In our case we assume that lysozyme amyloid fibrils could be destroyed by the partially uncovered magnetic nanoparticles with redox potential affecting chemical bounds in lysozyme amyloid fibrils. At present, it is not known whether Fe₃O₄ magnetite core is fully clamped inside apoferitin protein shell. However, we have demonstrated, that a partial destruction of apoferitin shell occurred, which can lead to partial exposure of magnetite core to the solvent. Thus, it follows from the experimental data, that the influence of magnetite core on anti-amyloid activity is a possibility that should be considered. We propose targeted transport and thus minimization of toxic effect of magnetoferritin nanoparticles on surrounding medium. Up to now, the effect of magnetoferritin on biological membrane/cells is still unknown. It is not excluded chemical surface modification of magnetoferritin nanoparticles for increasing its biocompatibility for various another applications. We are conscious that further characterization and possible therapeutic effect as well as cytotoxicity and possible side-effects on cell and animal models will be necessary to prepare magnetoferritin nanoparticles suitable for various bio-applications.
Figure 2. SAXS data and pair-distance distribution functions for magnetoferritin, lysozyme amyloid fibrils and their mixture in ratio 1:5. Figure taken from [12] with permission.

4. Potential bio-applications of magnetoferritin
Extensively studied physicochemical properties of magnetoferritin allow to use this unique magnetofarmaceutical nanomaterial in various application, as nanotechnology, industry or nanoelectronics (biosensors). The biocompatibility and high colloidal stability enhances the potential use in biomedicine [13] - [15]. In addition, magnetoferritin structure can be modified and functionalised by different chemical substances [16], drugs, surfactants, signal molecules or antibodies [17], [18]. Magnetoferritin can also serve as contrast agent in magnetic resonance imaging [19] or cell labeling [20], as a standard for diagnosis of various diseases [7] - [9], in cell separation [18], in hyperthermia therapy or in targeted transport of drugs [13] - [15]. Magnetoferritin characterised by peroxidase-like activity allows visualization of pathological tissues of the organism [21]. Recently it was shown, that magnetoferritin is able to decompose hydrogen peroxide in the presence of the substrate, N,N-diethyl-p-phenylenediamine sulfate, as evidenced by colour change after the reaction. This effect was stronger with loading factor increasing and could be useful in nanocatalytic chemistry [22]. Varying the synthesis technology, in combination with detailed fundamental research can advance and enlarge the range of potential applications of magnetoferritin.

5. References
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