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SANS contrast variation study of magnetoferritin structure at various iron loading

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A b s t r a c t

Magnetoferritin, a synthetic derivate of iron storage protein – ferritin, has been synthesized with different iron (Fe_3O_4-magnetite, $\gamma-Fe_2O_3$-maghemite) loading values. Small-angle neutron scattering experiments were applied to study the structure of magnetoferritin solutions using contrast variation method (various ratios of light and heavy water). Higher loading leads to increasing of the neutron scattering length density of magnetoferritin and increase of the polydispersity of complexes. The formation of the magnetic core and the variation of the protein shell structure are concluded.

1. Introduction

In 1992 Meldrum and co-workers prepared new magnetic material called magnetoferritin. Magnetoferritin differs from thewas derived from natural iron storage protein, ferritin, with difference in the iron core composition of magnetic iron oxide phasenanoparticles (Fe_3O_4, $\gamma-Fe_2O_3$) surrounded by the an empty protein shell – apoferritin with an outer diameter of about 12 nm [1]. Physico-chemical properties of magnetoferritin have been intensively studied. Bulte et al. studied magnetic properties of magnetoferritin using SQUID (superconducting quantum interference device) magnetometer and revealedresult shows superparamagnetic behavior of magnetoferritin dispersions [2]. Later, higher ratio of maghemite inside magnetoferritin was

detected using Mössbauer spectroscopy in magnetic field at 9 T and at temperature 4.2 K [3,4]. The study of Wong et al. confirmed the presence of superparamagnetic nanoparticles of Fe_3O_4 and γ-Fe_2O_3 in magnetoferritin, which was prepared by a new modify chemical process using a controlled amount of *oxidat*, ???? trimethylamine N-oxide, in anaerobic conditions, at temperature 65°C and pH 8.6. The new chemical method allowed to obtain magnetoferritins with various iron atoms per one apoferritin biomacromolecule (denoted as loading factor,: LF). By transmission electron microscopy the aggregation of magnetoferritin with LF > 1000 was observed. Results have shown that with higher LF the diameter of nanoparticles increases [5,6]. TIn 2007 the effect of dipolar interaction on temperature relaxation of magnetoferritin was studied and results were in a good agreement with theoretical models [7]. Kasyutich et al. in 2008 have prepared three-dimensional arranged crystals of magnetoferritin with size about 10 to 100 micrometers by protein crystallization technology. ~~These samples were characterized by Raman spectroscopy and SQUID magnetometry~~ (*not needed*)[8,9]. Afterward, magnetoferritin in this crystalline 3D arrangement was compared with amorphous material using small-angle X-rays and neutrons scattering and SQUID magnetometer [9]. Detailed experimental study of the magnetoferritin nanoparticles size in solution using transmission electron microscopy, X-ray diffraction, atomic force microscopy by Martínez-Pérez et al. showspoints that the external diameter of magnetoferritin nanoparticiles increased with LF. The reason of this effect was related to conformational changes of protein (apoferritin) due to binding of iron oxides [10]. Recent studies of magnetically induced optical birefringence (Δn) for colloidal dispersion of magnetoferritin in comparison with Δn of ferritin have shown differences in their magneto-optical behavior [11,12]. Magneto-optical Faraday rotation at room temperature in applied magnetic field with intensity H = 2970 Oe (i.e. 236.4 $kA.m^{-1}$ showed dDifferent spectral dependences of the Faraday rotation on the wavelength of light allowinged to distinguish ferritin and magnetoferritin. Dependence of the Faraday rotation of magnetoferritin as a function of applied magnetic field, characteristic for superparamagnetic system, could be modeledhas been described by the Langevin function with a log-normal size distribution, which allowed to obtain the diameter of the magnetoferritin core (size of magnetic particles within magnetoferritin molecules), about 6 nm [13]. Discovered The peroxidase-like activity of magnetoferritin was more pronounced in the case of higher LF. The results showed that magnetoferritin was able to decompose hydrogen peroxide in the presence of the substrate, N, N-diethyl-p-phenylenediamine sulfate, which changed the color after the reaction [14]. The physico-chemical characterization of magnetoferritin biomacromolecules in terms of morphology, structural and magnetic properties shows that iron oxides loaded into apoferritin affected the structure of protein shell [15,16]. However, the specific effect of magnetic nanoparticles and different amount of iron

inside one biomacromolecule (LF) on protein structure has not been fully determined yet.

Extensive studies of magnetoferritin from the first synthesis in 1992 showed that its biological origin, well-defined diameter of nanoparticles, colloidal stability and superparamagnetic behavior provide significant potential of magnetoferritin for application in nanotechnology, industry, but especially in the biomedical sciences and cell biology [1,17]. The ability offunction of magnetoferritin to undergo structure change under certain conditions enhance their potential for various applications. in possible application can be changed due to the destroyed structure depending on iron loading. Every application have some specific mechanism and Tthe magnetoferritin structure canould be modified by other substances [18], drugs, surfactants, signal molecules, antibodies [19,20], etc. resulting in change of the protein shell, so that the molecule can be completely closed, or partially open, or completely disrupted. And by this way could be the external structure more or completely closed. Various potential applications are reported throughBy different chemical modifications of the external or internal surface, such as it is possible to use magnetoferritin in magnetic resonance imaging of tissues [2,21], as contrast agent for cell labeling [22], as standard for diagnosis of various diseases [11-13], in nanockatalytical chemistry [14], cell separation [20] or in targeted transport of drugs. The drug binding into the magnetoferritin structure provides visualization of pathological tissues or targeted transport directly only to the damaged area of the organism without the side effects of the drug on healthy tissues and organs [23].

All these applications rely on the ability of magnetoferritin to pertain or to change its core-shell structure. For these reasons, magnetoferritin represents a suitable model system to study its structure by the small-angle neutrons scattering (SANS). Small-angle neutron scattering (SANS) is an experimental method particularly suited n experimental method to study the details of such core shell type objects of sizes 10-20 nanometers. that uses elastic neutron scattering at small scattering angles to investigate the structure of various substances at a mesoscopic scale of about 1 - 100 nm. Contrast variation technique in SANS can be used to reveal fine details of dispersions of multicomponent particles for systems of polydisperse, superparamagnetic non-interacting and interacting particles and clusters in liquid mediacarriers. The structure and interactions of magnetic zed or non-magneticzed particles can be obtained by the polarized neutron scattering analysis.

It is possible to investigate the atomic and magnetic structure of particles and the way they interact under various conditions. A versatile approach to study For polydisperse and nonhomogeneous particle dispersions by using contrast variation has been recently introduced systems the modified contrast is introduced as the difference between the effective mean

scattering length density and the density of the solvent. The shape scattering from the particle volume can be reliably obtained. [24,25]. Modifications of classical expressions describing changes in integral parameters of the scattering (forward scattering intensity, at zero angle, radius of gyration, Porod integral) with the contrast are can be analyzed and the shape and size polydispersity is calculated. . In comparison with the monodisperse case, the residual scattering in the minimum of intensity as a function of contrast (effective match point) in polydisperse systems makes it possible to treat the Guinier region of scattering curves around the effective match point quite precisely from the statistical viewpoint. The effect of magnetic scattering in small-angle neutron scattering from superparamagnetic nanoparticles could be obtained using the basic functions approach or by the modifications of the integral parameters in the case of polydisperse multicomponent particles [24,25]. In this work, we have investigated the structural changes of magnetoferritin with various loading factors LF by SANS contrast variation method. Results have shown the increase of effective match points and polydispersity of magnetoferritin with the LF growth.

2. Materials and methods

2.1 Chemicals

Ammonium ferrous sulfate hexahydrate ($(NH_4)_2Fe(SO_4)_2.6H_2O$), equine spleen apoferritin in 0,15 M NaCl, 3-[(1,1-dimethyl-2-hydroxy-ethyl)amino]-2-hydroxylpropane-sulfonic acid (AMPSO), hydrogen peroxide (H_2O_2), hydrochlorid acid (HCl), potassium thiocyanate (KSCN), sodium hydroxide (NaOH) and trimethylamine N-oxide (Me_3NO) were obtained from SIGMA-Aldrich.

2.2 *Magnetoferritin preparation*

Magnetoferritin was synthesized by incorporation of ferrous ions into the apoferritin hollow sphere in 0.05 M AMPSO, pH 8.6 and anaerobic conditions at 65°C. Magnetoferritin complexes with four loading factors (LF) of 160, 510, 766 and 910, representing the average number of iron atoms per one apoferritin cage, were prepared. The LF was determined spectrophotometrically using UV-VIS spectrophotometer SPECORD 40, Analytik Jena. LF`s values are found with errors less than 1%. Concentration of iron was measured after HCl/H_2O_2 oxidation and KSCN addition by light absorption of thiocyanate complex at wavelength (λ) 400 nm. Protein amount was detected using standard Bradford method at λ = 595 nm. After the synthesis samples were 24 h freeze dried to obtain a powder. Finally, 10 mg/ml solutions of protein were prepared by dissolving powders with various ratio of H_2O:D_2O for contrast

variation method. AMPSO alkaline buffer solution as background was prepared by dissolving of AMPSO (Sigma) in accurate ratio of H_2O:D_2O.

2.3 Small-angle neutron scattering experiment

SANS measurements were carried out on small-angle neutron scattering spectrometer YuMO [26,2717,18] installed on a pulsed reactor IBR-2 at the Joint Institute for Nuclear Research (JINR, Russia). Samples were located in standard quartz cuvettes (Hellma) with a thickness of 1 mm and experiments were performed at room temperature with the scattering of thermal non-polarized neutrons (wavelength from 0.5 Å to 8 Å) in the absence of external magnetic field. Measured scattering curves were corrected for background effects (AMPSO with water buffer) and absolute scaling of the spectra were done cross section of neutrons was made by vanadium calibration standards.

3. Results and discussions

The small-angle neutrons scattering from un-magnetized magnetoferritin aqueous suspension was measured at the different D_2O content in the solvent. Experimental scattering curves for apoferritin (concentration about 12 mg/ml) and magnetoferritin with LF 160, 510, 766, and 910 in 100% D_2O, after substraction of scattering from AMPSO buffer, are shown in Fig. 1. Some smoothing of the measured experimental SANS curve in comparison with pure apoferritin is seen like in previous works [15,16]. Similar behavior of SANS curves at varying H_2O/D_2O content is obtained for all studied LFs of magnetoferritin except LF 910. For magnetoferritin with LF 910 dark precipitates sedimented was observed toat the bottom of the cell.and colorless liquid system in the top. Therefore, at`s why only MFer 910 in 100% D_2O was measured. For example, SANS curves of magnetoferritin with LF 766 in various ratios of H_2O and D_2O with protein concentration of 10 mg/ml are shown in Fig 2 a.). From linear fitting of Guinier plots (Fig. 2 b.)) values of forward scattering intensity, I_0, and radius of the gyration, R_g were obtained. Also scattering length densities (SLDs) of the solvent mixtures, ρ_s, were calculated using the SLD of H_2O and D_2O values (ρ_{H2O} = -0.559. 10^{10} cm^{-2}, ρ_{D2O} = 6.34. 10^{10} cm^{-2}). The lowest value of zero-angle scattering intensity of magnetoferritin dispersion was seen in the match point. For homogeneous particles, theAs it is well known scattering length density of the solvent and particles coincides in the match point. Non-zero value of the forward scattering intensity in the match point is seen in Fig. 3 , indicatingand it`s pointed out to the structural polydispersity in the system. The It was observed that match point is shifted with increasing of loading factor (Fig. 3). The Neutrons magnetic scattering contribution can be neglected for the considered LFs (contribution of magnetic scattering for LF 766 is less than 5%). Already in the

monodisperse approximation under precondition that the magnetic core in magnetoferritin consists of magnetite the shifts of the effective match points (corresponding SLDs are 2.46. 10^{10} cm^{-2}, 2.79. 10^{10} cm^{-2}, and 2.86. 10^{10} cm^{-2} for LF 160, and LF 510 and LF 766, respectively) as compared to the protein moiety of apoferritin (SLD 2.34. 10^{10} cm^{-2}) give 0.026, 0.099 and 0.114 respectively, for the volume fractions of magnetic material in the magnetoferritin cage. These valuesparameters are larger than the iron content calculatedobtained from the synthesis parametersprocess (0.005, 0.017 and 0.025 for the LF 160 and LF 510 and LF 766, respectively). The results do not differ by less then by 5% in the case of maghemite instead of magnetite. The calculated match points are significantly higher than those for native ferritin with different amount of iron [2819]. The obtained match point of magnetoferritin corresponds to native ferritin with about 800 and 2000 iron atoms per protein shell. Thus, it was observed from SANS contrast variation measurements indicated abnormally high ratio of between the amount of magnetic material to the and protein in the structure of magnetoferritin. The reason of this increase canould be explained well by the partial destruction of the shell, and thus the effective growth of the relative content of the magnetic component in thehe structure of magnetoferritin. The residual scattering in the effective match points which is in accordance with polydispersity increases in accordance withthus confirming the widening of the polydispersity function for larger LFs.

The precise mechanism of magnetic loading influence on the protein structure is not known yet. In a recent As it was previously studytudied the apoferritin disassembly was observed at pH below 3.4 [2920]. , This is which, however, is not our case, since our magnetoferritin is prepared in alkaline pH (pH 8.6 for each synthesis). While it was not possible to control pH directly during the synthesis process due to the hermetically enclosed reaction bottle, still after the synthesis the pH value was checked and only slight pH change, namely some decrease for higher LFs, was detected but the minimum pH after the synthesis was above 7 for all LFs.

The observed disassembly of magnetoferritin cage can be related to the influencespecific effect of the magnetic nanoparticles. Understanding of the interaction between magnetic nanoparticles and proteins is the subject of extensive studies in recent years and the general conclusion is that different nanoparticles with specific concentration, surface and size can affect proteins variously [30-3421-25].

4. Conclusions

In the SANS experiment Wwe studied the structural changes of magnetoferritin in aqueous solution with LFs: 160, 510, 766 and 910. The obtained values of average neutron scattering length density of magnetoferritin (effective match point) and it´s variation among the

aggregates increased with iron loading. It could be associated with formation of magnetic particles inside complexes and their influence on the protein structure.

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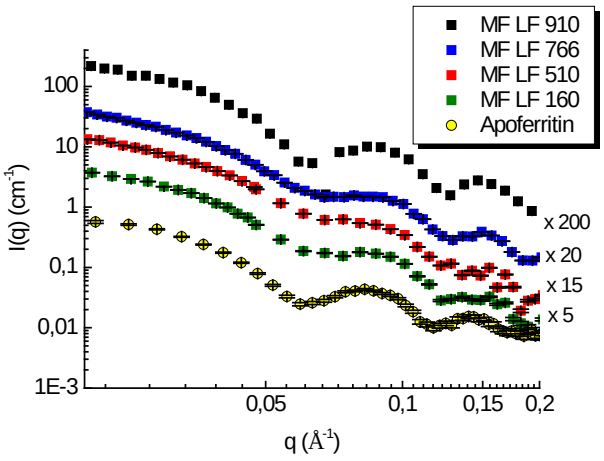

Fig. 1 Comparative experimental scattering curves of magnetoferritins (LF 160, 510, 766, 910) and apoferritin in 100% D_2O with protein concentration of about 12 mg/ml for apoferritin and 10 mg/ml for magnetoferritin samples.

a.) b.)

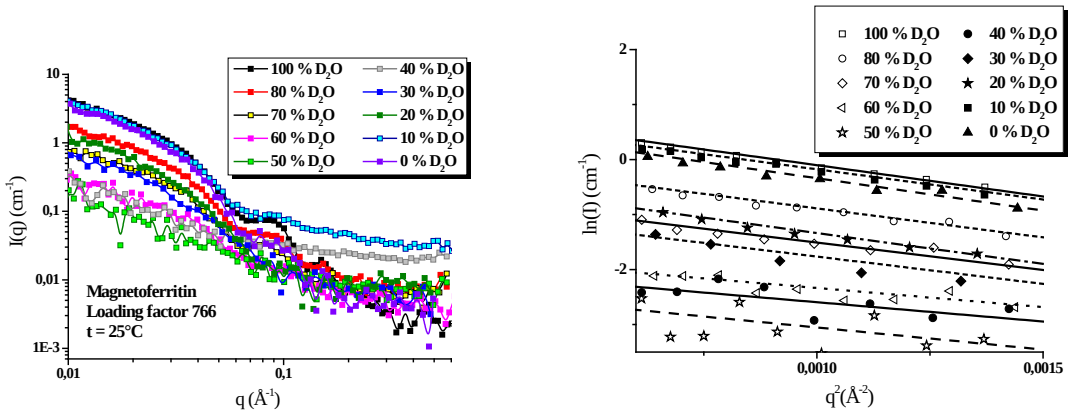

Fig. 2 Scattering curves of magnetoferritin LF 766 in various ratios of H_2O and D_2O with protein concentration of 10 mg/ml (a) and corresponding Guinier plots for SANS contrast variation data from magnetoferritin with LF 766 (b).

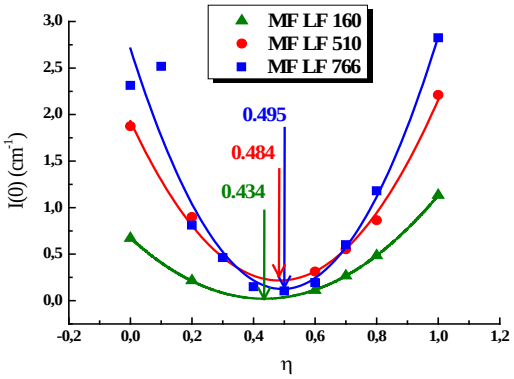

Fig. 3 Graph of the scattering intensity at the zero angle as a function of the D_2O content - η. The resulting effective match point ($\eta_{MF\ LF\ 766}$ = 0,495) for magnetoferritin with LF 766 (blue square) is compared with data for magnetoferritin with LF 160 (green triangle) and 510 (red circle).