Journal of Magnetism and Magnetic Materials 471 (2019) 400–405

Contents lists available at ScienceDirect

Journal of Magnetism and Magnetic Materials

journal homepage: www.elsevier.com/locate/jmmm

Research articles

# Self-assembly of hen egg white lysozyme fibrils doped with magnetic particles

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ARTICLE INFO

*Keywords:*
Magnetic nanoparticles
Amyloid fibrils
Self-assembly
Birefringence

ABSTRACT

In this work, the interaction between spherical magnetic nanoparticles and amyloid fibrils as well as formation of self-assembled structures during drying process of such colloidal system were investigated. The final self assembled structures were observed experimentally by atomic force microscopy, scanning electron microscopy and polarizing microscopy. Our results show that the colloidal composites are self-assembled into flower-like patterns after the evaporation of liquid drop. These observations show that a large scale and well-organized multi-branched aggregates are formed during the process of drying. Moreover, observation by polarizing microscopy under crossed polarizers showed birefringence.

## 1. Introduction

Self-assembly of molecules is obvious in natural systems and therefore it is basis for the formation of diverse and complex biological structures [1]. There exists many ways how to obtain self-assembly or various patterns formation process onto a solid surface [2,3]. This effect may be either due to direct adsorption of the particles on the surface [4–6] or it can be the result of the evaporation of the solvent from the liquid drop [7–9]. Evaporation of solvent can be an important access to induce kinetically stable assemblies of building blocks with a large-scale specific arrangement [10]. In nature the process of self-assembly is governed by intra- and inter-molecular forces that lead molecules into a stable and low energy state. The mentioned forces include electrostatic interaction, hydrogen bonding, hydrophophic interactions and van der Waals forces. By combination of these forces together as a whole, it is possible to obtain structural conformation of all biological macromolecules and influence their interaction with other molecules as well [1]. The structure of the deposit can have various patterns from coffee drops [11] to more complex aggregates like fractal patterns [12] due to the physical and chemical processes that are involved during evaporation. Proteins are known to form highly complex of self-assembly patterns, for example: concentric rings, treelike fractals or dendrites by using salt-induced molecular self-assembly and droplet evaporation methods [13]. Understanding the mechanism of supramolecular assembly of small particles to form larger structures leads to potential application in materials engineering, where self-assembly into well-structured complexes can be used to fabricate advanced materials.

Amyloid fibrils have an important role in nanotechnology and biomaterials applications due to their unique physical and mechanical properties [14,15]. The most used and well characterized model protein for *in vitro* study of amyloid fibrillation is Hen egg white lysozyme (HEWL) that represents a structural homologue of human lysozyme. Magnetic nanoparticles (MNPs) are currently used in biomedical applications such as magnetic resonance imaging, drug delivery or even hyperthermia [16–19]. Nanoparticles may influence not only the formation, but also the structural stability of protein amyloid aggregates. The powerful tool is using MNPs and magnetic field as an organizing medium to induce the assembly of amyloid fibrils. Bolisetty and coworkers [20] have demonstrated the possibility to orient amyloid fibrils of $\beta$-lactoglobulin with MNPs under an external magnetic field due to adsorption of magnetic nanoparticles on fibril surface. In our previous works [21,22] the adsorption of MNPs on lysozyme amyloid fibrils (LAF) was observed where the adsorption was concentration dependent. The controlled self-assembly of lysozyme fibrils into micro-scale

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https://doi.org/10.1016/j.jmmm.2018.09.109
Received 12 June 2018; Received in revised form 19 September 2018; Accepted 28 September 2018
Available online 29 September 2018
0304-8853/ 

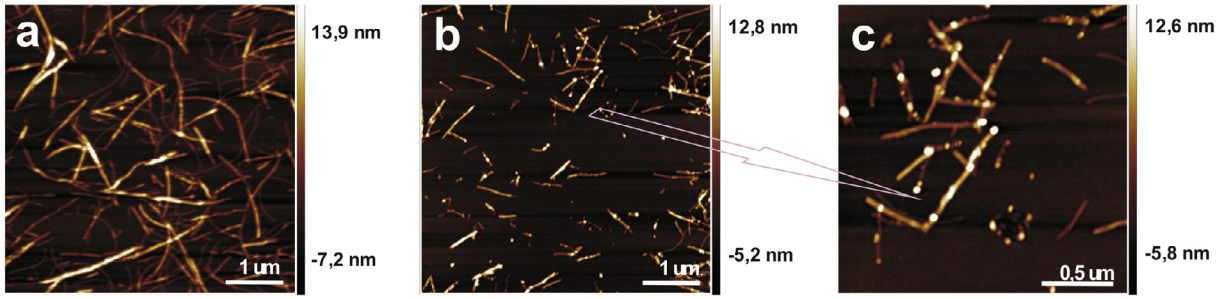


**Fig. 1.** Representative AFM images of (a) pure amyloid fibrils and (b) amyloid fibrils with magnetic nanoparticles, (c) the detail of the image (b).

structure with magnetic nanoparticles under the application of magnetic field is crucial for the study of their collective properties. Magnetic nanoparticles as a template for self-assembly of lysozyme fibrils represent an important tool for the achievement of various MNPs-based facilities [23]. The dynamic of forming dendritic structures during drying process was studied in our previous work by N. Tomasovicova, et al. [24]. In this work, we report detailed study of flower-like dendritic structures obtained by self-assembled suspension of lysozyme amyloid fibrils with the magnetic nanoparticles. The final structure was observed by polarizing microscopy (PM), atomic force microscopy (AFM) and scanning electron microscopy (SEM). These experimental techniques were used for better characterization of topography and morphology of dendritic structure as well as their building blocks. The influence of salt on the formation of dendritic structure as well as optical properties of such bio-hybrid structure were also studied.

## 2. Materials and methods

Hen egg white lysozyme (HEWL) (lyophilized powder, lot number L6876, 50,000 units mg 1 protein) was obtained from Sigma-Aldrich Chemical Company (St Louis, MO). All other chemicals were obtained from Sigma or Fluka and were of analytical reagent grade. Lysozyme amyloid fibrils were prepared by dissolving of HEWL powder to obtain a final concentration of 10 mg/ml in 0.2 M glycine-HCl buffer with pH 2.2 and 80 mM NaCl. Prepared solution in enclosed bottle was heated for 2 h at 65 °C with constant stirring speed of 250 rpm.

The magnetite nanoparticles were prepared by co-precipitation method [25]. The synthetized magnetic nanoparticles were washed by magnetic decantation several times to remove impurities produced during the precipitation process. After that the nanoparticle dispersion was stabilized by addition of perchloric acid; in acidic medium $Fe_3O_4$ nanoparticles are positively charged and $ClO_4^-$ species are adsorbed on their surface. This ensures the electrostatic repulsion between the nanoparticles and thus the stability of prepared magnetic fluid. Electrostatically stabilized MNPs were dispersed in water to obtain magnetic fluid (MF). The mean hydrodynamic diameter of nanoparticles $d$ = 26 nm was determined by dynamic light scattering using Zetasizer Nano ZS by Malvern Instruments (Germany). The value of polydispersity index was 0.26. Such magnetic fluids were used for preparation of mixture with amyloids.

Magnetic fluid with concentration of MNPs of 28 mg/ml was added to the initial solution of lysozyme amyloid fibrils (sLAF) with concentration of 10 mg/ml to achieve a ratio between volume of sLAF and MF solutions ($V_{sLAF}$: $V_{MF}$) in the mixture: 1 ml: 0.1 ml. The final concentration of MNPs in prepared solution was 2.8 mg/ml.

The $\zeta$-potentials for individual sample were measured by Laser Doppler Electrophoretic measurement using Zetasizer Nano ZS by Malvern Instruments (Germany). Measurements were done at temperature 20 °C.

AFM topographic images of sLAF and mixture of sLAF and MNPs on mica were acquired in tapping mode at ambient conditions with a commercial beam-deflection AFM (Veeco di Innova, Bruker AXS Inc., Madison) using silicon AFM probes with a gold coating of the cantilever backside. The samples were prepared by drop casting of solution on the surface of freshly cleaved mica. They were rinsed with ultrapure water after 5–10 min of adsorption to remove redundant sample. Then the samples were left to dry before scanning.

Optical imaging, AFM and SEM observation of dendritic structures obtained by drying process were performed on the same sample. The solution was dropped on the glass slide and left to dry. Dried sample was observed first by optical microscopy followed by AFM. Before SEM imaging, a thin layer of Au was applied on prepared sample. Optical imaging was observed with the polarizing microscope Nikon Eclipse LV100 under bright field, using lenses of 10×, 20× and 50× magnifications. AFM observation was performed using Agilent 5500 AFM system equipped by PicoView 1.14.3 control software. The topography images were acquired in the tapping mode with standard silicon cantilevers with nominal resonant frequency of 300 kHz and nominal spring constant of 26 N/m (Olympus, model OMCL-AC 160TS). Images were processed using freely available software from Gwyddion ( http://gwyddion.net). Scanning electron microscope (SEM) Tescan-VEGA3 LMU was used for morphological description of sample to see the structures at higher resolution. All measurements were carried out at ambient temperature in air, while relative humidity was in the range of 30–40%. The polarization dependent experiments were carried out by the same polarizing microscope with the possibility to vary the polarizer axis by 360° in order to analyse the transmitted intensity as a function of angle between the polarizer axis and orientation of the structures. The sample was diluted ten times and deposed on the glass slide.

## 3. Experimental results and discussion

Fig. 1 shows AFM images of pure sLAF and sLAF doped with MNPs. From the AFM images, it can be seen the typical amyloid morphology that confirm strongly the elongated character of the studied aggregates. The adsorption of MNPs on LAF clarifies interaction activity between these two components. The study of interactions of different nanoparticles such as iron oxide, Ag and Au with different proteins as $\beta$-lactoglobulin, insulin and lysozyme [26–28] showed the adhesion of nanoparticles on the fibril surfaces. These results are in excellent agreement with our results and can be observed in bulk samples.

The forces of electrostatic interaction as well as van der Waals represent two types of binding forces that take action in the interplay between the protein fibrils and MNPs [29]. The attractive protein-protein interactions should exist to initiate the self-organization of proteins. The isoelectric point, that represents the pH value at which the $\zeta$-potential value is zero due to the no electric charge on the surface of a particle, of HEWL is 11.35 and therefore, at pH 2.2 the protein is positively charged. The positive $\zeta$-potential for our sLAF of 38.7 mV has been confirmed. Accounts on that, the protein molecules repulse each other due to the same-charge exclusion effect. However, different situation is in the case of $Fe_3O_4$ MNPs, where the $\zeta$-potential depends on pH. The positive $\zeta$-potential was observed at pH<6.3, while at pH>6.3 is