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Dynamic morphogenesis of dendritic structures formation in hen egg white lysozyme fibrils doped with magnetic nanoparticles

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ABSTRACT

In this research, the dynamic process of aggregation that forms microflower morphology in solution of lysozyme amyloid fibrils doped with spherical or spindle-like magnetic nanoparticles during the process of drying as well as their final microstructures were investigated. The prepared lysozyme amyloid fibrils as well as their mixtures with in-lab synthesized magnetic particles, which were prepared by adding the nanoparticles to the fibrils solution after the process of fibrillation was done, were characterized using brightfield trans-illumination-mode optical microscope, atomic force microscopy (AFM) and scanning electron microscope (SEM). Brightfield optical imaging bases upon photoabsorptive property of the fibrils-nanoparticle composites clearly reveals the morphological features in microscale, and additionally, for the *in vivo*, live action of the time-dependent process of self-assembly of such composites composed of fibrillary structure incorporated with magnetic particles was optically elucidated at ambient temperature. Moreover, while results of AFM reveal delicate and peculiar association of fibrils with magnetic nanoparticles of different shapes, SEM images illustrate a stark difference in fine detailed final morphology of microstructures associated with spherical and spindle-like nanoparticles. Our results indicated that the interaction between fibrils solution and the nanoparticles commence right after mixing, the dynamic process of forming dendritic structure resembling microflower morphology is on the order of minutes, and its final structure is highly dependent on the shape of magnetic nanoparticles.

1. Introduction

Currently, one of the hot research topics around the world is to design nanomaterials that are capable of assembling into multifunctional superstructures. A variety of liquid crystals (LCs) themselves are prominent examples of materials in which organized self-assembly appears spontaneously on different scales. Besides the local ordering on the molecular level, they may form micro/macroscopic superstructures. Self-assembly of molecules is the fundamental basis of the formation of diverse and complex biological structure in all systems of living organisms [1]. For instance, structure of amyloid fibrils is an interesting model for elucidating liquid crystal phase behavior of biological anisotropic colloidal suspensions [2–5] which have been extensively studied for its consequential relation with many human neurodegenerative disorders such as Alzheimer's disease and Hungtinton's disease [6–9]. In particular, lysozyme amyloid fibrils are formed by self-assembly process, and can be recognized as the highly ordered nanoscale assemblies of protein protofibrils with characteristic cross-stacking perpendicular to the length axis of the fiber [9].

Magnetic nanoparticles (MNPs) have shown its remarkable functional versatility such as superparamagnetism, high field irreversibility, high saturation field that make them very attractive for applications in biomedicine such as in drug targeting delivery, magnetic hyperthermia, arrangement of biological assemblies, contrast agents in MRI, biomagnetic separation [10]. Nominally, biomedical applications require the magnetic particles to be stable in water at neutral pH and physiological salinity. The colloidal stability of magnetic fluids depends on the dimensions of particles, which should be sufficiently small to avoid agglomeration as well as the binding to each other on surfactant coating, commonly a monolayer of oleic acid (steric repulsion), or otherwise, particles have to be prevented from sticking to each other by electrostatic layer via electrostatic repulsion [11–15]. Furthermore, for *in vivo* applica-

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tions the magnetic particles must be coated with biocompatibile polymer in order to render specific functionality [16,17]. Potential use of functionalized MNPs for addressing Alzheimer's disease was demonstrated in works [18,19]. For example, a research group from [20] chose iron-oxide nanoparticles composed of different core materials such as magnetite and maghemite. Different molecular coatings such as dextran, carboxydextran, polystyrene alongside different hydrodynamic diameters have been applied for internalization into U118 glioma cells and HUVEC, which were monitored after 12, 24, 36 and 48 h. This work demonstrated that cells confer different amount of uptake of iron oxide nanoparticles, potentially useful for diverse applications.

Development of such bio-inorganic hybrid nanomaterials with advanced properties that improve performance is receiving considerable interest due to the possibility of achieving biocompatibility and triggering novel nanotechnological applications. Much endeavor has been devoted to synthesizing biotemplated magnetic nanomaterials not only attributing to their potential use in medical diagnostics as contrast agents, biosensing and therapeutic agents, but also for their relevance in high-density data storage units. Particularly, magnetic hybrids offer potential capability to achieving high spatial order and alignment which is a highly desired feature in many applications. In research reported by [21] β-lactoglobulin was doped with Fe_3O_4 magnetic nanoparticles, and the results of such combination indicate that depending on the pH of the solvent used in synthesis, different hybrid aggregates such as nanoparticle-modified amyloid fibrils and spherical nanoclusters can be obtained. More recently, Chen et al. [22] have studied the effect of Fe_3O_4 MNPs on fibrillation process of insulin proteins. The authors confirmed that no effect on the conformational change of insulin fibrils was observed when increasing concentration of magnetic nanoparticles were applied to the fibrillary structure. Also, in both aforementioned work, the magnetic nanoparticles were added to the solution during fibrillation process, which demonstrated strong adsorption of magnetic nanoparticles onto protein amyloid fibrils. Moreover, in the aspect of functionality, the hybrid structures demonstrated in [21] have the ability to be aligned by the influence of external magnetic field. Additionally, as confirmed empirically in [22,23][22,23], the solution of mixture of magnetic nanoparticles with insulin or lysozyme amyloid fibrils remains stable several weeks after sample preparation. Likewise in work [24] it concluded that functionalized magnetite nanoparticles can aggregate and form nanoflower morphology, and their application as a T2 magnetic resonance imaging (MRI) contrast agent was evaluated.

One of the key tasks of the paper herein was to prepare bio-inorganic hybrids-based LCs formed by lysozyme fibrils alongside doping of magnetic nanoparticles, and to study the interaction between magnetic nanoparticles and lysozyme amyloid fibrils as well as their consequential structure. Additionally, in the context of previous research, time-dependent process of morphological changes of amyloid fibrils and their self-assembly was visualized and characterized with brightfield trans-illumination mode optical microscope.

2. Experimental methods

2.1. Sample preparation

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 5 mg/ml in 0.2 M glycine–HCl buffer with pH 2.2 and 80 mM NaCl (LAF). Prepared solution in enclosed bottle was heated for 2 h at 65 °C with constant stirring speed of 250 rpm.

The spherical magnetic nanoparticles were prepared by co-precipitation of Fe^{2+} and Fe^{3+} salts by NH_4OH at 60 °C [25]. The precipitate was isolated from the solution by magnetic decantation with distilled water. Then the freshly prepared magnetic nanoparticles were dispersed in water and electrostatically stabilized with $HClO_4$ to obtain magnetic fluid (MF) with MNP concentration of 28.27 mg/ml. The mean hydrodynamic diameter of MNPs of 26 nm was estimated by dynamic light scattering (Zetasizer).

For the synthesis of spindle-like hematite (α-Fe_2O_3) nanoparticles it was proceeded according to the protocol presented by Ozaki et al. [26], and all chemicals were used without any purification. The iron chloride ($FeCl_3 \cdot 6H_2O$, 26 mmol) with $NaH_2PO_4 \cdot H_2O$ (c = 0.8047 mmol) were dissolved in 1 l of water at 90 °C in three-neck round bottom flask, and the reaction solution was heated up to boiling point and refluxed for 96 h. After synthesis the sample was washed three times with water and centrifugated at 7000 rpm for 10 min; afterwards, the size separation from base sample was done by several times centrifuging at 2000 rpm, 5000 rpm and 6000 rpm. The prepared nanoparticles were caped with phosphate acid and stabilized in polar solvents, and the concentration of magnetic particles in solution was 8.4 mg/ml.

The mixture of LAF with magnetic particles was prepared by adding the MF to the solution of LAF. The final concentration of magnetic particles in the mixture was 56 μg/100 μl for spherical magnetic particles and 24 μg/100 μl for spindle magnetic particles.

2.2. Zeta potential analyzer

To examine the zeta potential of magnetic nanoparticles, lysozyme fibrils and their mixture, a zeta potential analyzer was employed. The zeta potential was measured at 25 °C with nanoZS Zetasizer (Malvern).

2.3. Scanning electron microscopy

The morphology and size of the spindle nanoparticles were obtained by scanning electron microscopy (SEM). The particle size distribution was obtained by manually measuring length, diameter and aspect ratio of at least 100 nanoparticles, and the mean values of these parameters were obtained by using the log-normal distribution function. The dried samples from optical experiment were used for observing the dendritic structure by SEM measurements; for morphological description the dried Au- coated samples were observed by Tescan-VEGA3 LMU SEM.

2.4. Atomic force microscopy

Samples for atomic force microscopy (AFM) were prepared by drop-casting of solution on the surface of freshly cleaved mica and after 5–10 min adsorption they were rinsed with ultrapure water added dropwise to remove redundant sample. Then the samples were left to dry before scan. AFM topographic images were acquired by tapping mode in ambient condition with a commercial beam-deflection AFM (Veeco di Innova, Bruker AXS Inc., Madison). The AFM probes used throughout the AFM experimental session was Si-tip with backside Au-coated cantilever (Nanosensors, PPP-FMAuD-20, K 0.5–9.5 N/m, nominal tip height 10–15 μm).

2.5. Brightfield trans-illumination-mode optical microscope

Based upon the photo-absorptive property of magnetic nanoparticle-incorporated lysozyme fibrils, custom-built brightfield trans-illumination-mode optical microscope was utilized to revealing the time-resolved structural morphogenesis of lysozyme