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Interaction of magnetic nanoparticles with lysozyme amyloid fibrils

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This work is devoted to the structural study of complex solutions of magnetic nanoparticles with lysozyme amyloid fibrils due to possible ordering of such system applying the external magnetic field. The interaction of magnetic nanoparticles with amyloid fibrils has been followed by atomic force microscopy and small-angle X-ray scattering. It has been observed that magnetic nanoparticles (MNPs) adsorb to lyzozyme amyloid fibrils. It was found that MNPs alter amyloids structures, namely the diameter of lysozyme amyloid fibrils becomes less define and decreases whole fibrils length, in same time MNPs do not change the helical pitch significantly,.

1. Introduction

Amyloid fibrils have an important role in nanotechnology and biomaterials applications due to their unique physical and mechanical properties [1,2]. Also amyloid fibrils, highly ordered nanoscale assemblies of protein protofibrils with characteristic cross- stacking perpendicular to the long axis of the fiber [3] are associated with various neurodegenerative diseases. Thus detail study of amyloids is of great interest. The most used and well characterised model protein for *in vitro* study of amyloid fibrillation is Hen egg white lysozyme (HEWL) that represents a structural homologue of human lysozyme with globular shape. While native lysozyme is a relatively stable enzyme under physiological conditions and does not easily form amyloids, mutant versions are implicated in some forms of hereditary systemic amyloidosis in humans [4]. Therefore forming amyloids from HEWL *in vitro* requires the use of specific environmental conditions, as high concentrations in solutions under acidic pH, constant stirring, high temperature and ionic strength or presence of various salts [5]. In was shown, that the kinetics of fibrils formation under these conditions is concentration dependent [6]. Fibrils may show several polymorphic states such as twisted ribbons, helical ribbons or nanotubes [7]. The coexistence of twisted and helical ribbons has been reported in the case of egg lysozyme after long incubation time [8]. It was observed that there exist some time- dependent transformations of twisted ribbon to nanotubes thought the helical ribbon intermediated [9].

Except the study of amyloid fibrils, nowadays there is increasing interest of magnetic nanoparticles (MNPs) as novel materials very suitable for biomedical uses such as hyperthermia or magnetic resonance imaging, drug delivery etc. Zaman et al. suggested existence of critical concentration ($c_{critical}$) at which amyloid fibrils are ordered to pure biological liquid crystal (LC) [10]. We propose to add magnetic nanoparticles into the solution containing lysozyme fibrils at lysozyme concentration lower than critical concentration $c_{lyz} < c_{critical}$. Our expectation is that magnetite nanoparticles will interact with lysozyme amyloid fibrils. Main aim is to study the fibrils-magnetic nanoparticles complexes if they may be oriented below $c_{critical}$ by applying the external magnetic

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field in order to obtain a biological liquid crystal. In our recent work the adsorption of MNPs on amyloid fibrils of HEWL in 2 mg/mL acidic solutions has been detected for the MNPs concentration range of 0.01-0.1% [11]. It has been observed that adsorption is determined by the MNPs content and aggregates of the MNPs follow the rod-like structure of fibrils at high MNP concentrations. However, the mechanism of such nanoparticle effect on protein amyloid aggregation has not been explained yet. Several researches show that different size, surface and concentration of nanoparticles affect protein aggregation in different way [12,13]. Therefore, the main goal of present research is the detail study and understanding the interaction between MNPs and solution of LAF with protein concentration of 10 mg/ml that is much higher as it was used in previous experiments with LAF. It should be mentioned that slightly different procedure for amyloids formation was used in this work. The inner structural organization of initial fibrillized amyloid solution and its mixtures with MNPs were analysed by means small-angle X-ray scattering (SAXS) and atomic force microscopy (AFM).

2. Materials and Methods

Hen egg white lysozyme (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 (LAF) were prepared by dissolving of lysozyme powder to a final concentration of 10 mg/ml in 0.2 M glycine-HCl buffer with pH=2.4 and 80 mMNaCl. Prepared solution in enclosed bottle was heated for 2 hours at 65°C and constant stirring (250 rpm). The magnetite nanoparticles were prepared by co-precipitation method [14]. Electrostatically stabilized magnetic nanoparticles with $HClO_4$ were dispersed in water to obtain magnetic fluid with mean diameter of nanoparticles d = 26 nm. The size of MNPs was determined by dynamic light scattering (Zetasizer). Such magnetic fluids were used for preparation of mixtures with amyloids.

Magnetic fluid (MF) with concentration of MNPs of 45 mg/ml was added to the initial solution of LAF (sLAF) with concentration of 10 mg/ml to achieve three different ratios between volumes of sLAF and MNPs solutions (V_{sLAF}:V_{MF}) in the mixture: 1ml: 0.0023ml, 1ml: 0.011ml, 1ml: 0.023 ml to obtain final mixture with mass ratio 10:0.1, 10:0.5 and 10:1, respectively.

The SAXS experiments were conducted on a Nanostar (Bruker AXS GmbH, Karlsruhe, Germany) laboratory device with the following characteristic parameters: microfocus X- ray souce IμS, CuKα wavelength of 1.54 Å, detector VANTEC-2000 (14 x 14 cm in size, resolution of 2048 x 2048 pixels). The samples were placed into glass capillary vessels with a diameter of 2 mm. The measurements were performed at 25±1 °C. The primary processing of data with taking into account the scattering from the solvent and capillary, detector efficiency, and background signal, as well as the recalculation to the absolute cross section was performed with the SuperSAXS (Jan Pedersen&Cristiano Oliveira) code.

Atomic force microscopy measurements were performed using Agilent 5500 AFM system equipped by PicoView 1.14.3 control software. The topography images were acquired in the tapping mode with a standard silicon cantilevers (Olympus, model OMCL-AC 160TS) with resonant frequency 300 kHz (typ.), and spring constant 26 N/m (typ.). All measurements were carried out at ambient temperature in air, while relative humidity was in the range of 30-40%. Images were processed using freely available software from Gwyddion (http://gwyddion.net). Samples for AFM observations were prepared by dropping the sample on freshly cleaved pieces of mica (PELCO® Mica Sheets Grade V5, 15 X 15 mm^2). After the deposition, the drop was left to incubate for 5-10 minutes, and then removed by rinsing the surface of mica substrate by ultra-pure water. Samples were dried in air at ambient temperature (typically for 1 hour).

3. Experimental results

Fig. 1 and Fig. 2 show AFM images of pure lysozyme fibrils and those doped with magnetic

fluid, respectively. The doped sample for AFM was prepared with ratio of SLAF volume to magnetic fluid volume (sLAF/MF= 1:0.023). The doped sample was deposed on mica 1 hour after preparation. As the volume concentration of magnetic particles is high, it is seen that some fibrils are completely covered by magnetic particles (see e.g. Profile 4 in Fig. 2), however it is clearly seen, that in sample are present also fibrils covered only with few particles as well as without particles (as reveals Profile 3 in Fig. 2). If compare Fig. 1 and Fig. 2 it is clearly shown that after doping with MF the length of fibrils is shorter. AFM measurements also reveal presence of a helical structure. The obtained diameter of the fibrils in initial solution is 7-9 nm. In the doped sample with MNPs the diameter is 9-10nm.

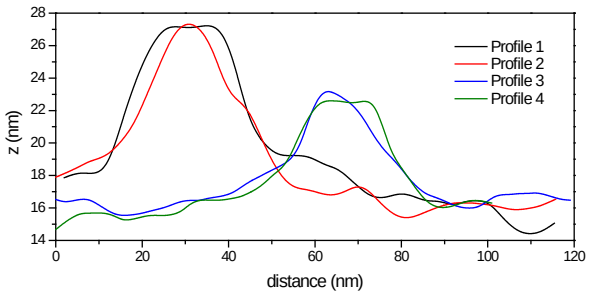
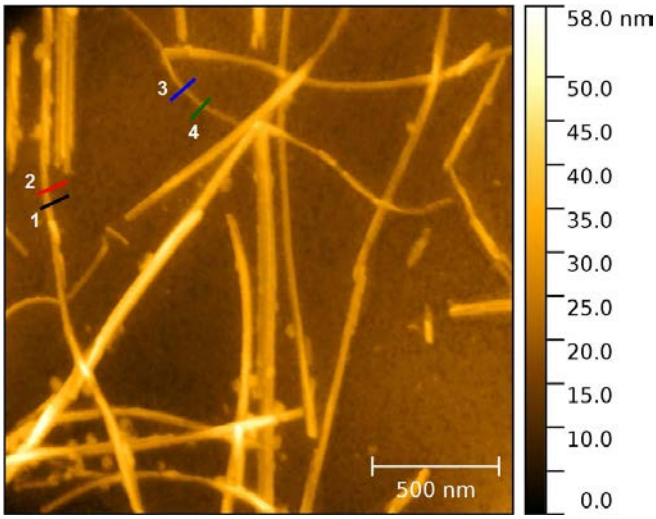


Fig. 1. AFM height image and their representative profile cross section for pure lysozyme fibrils.



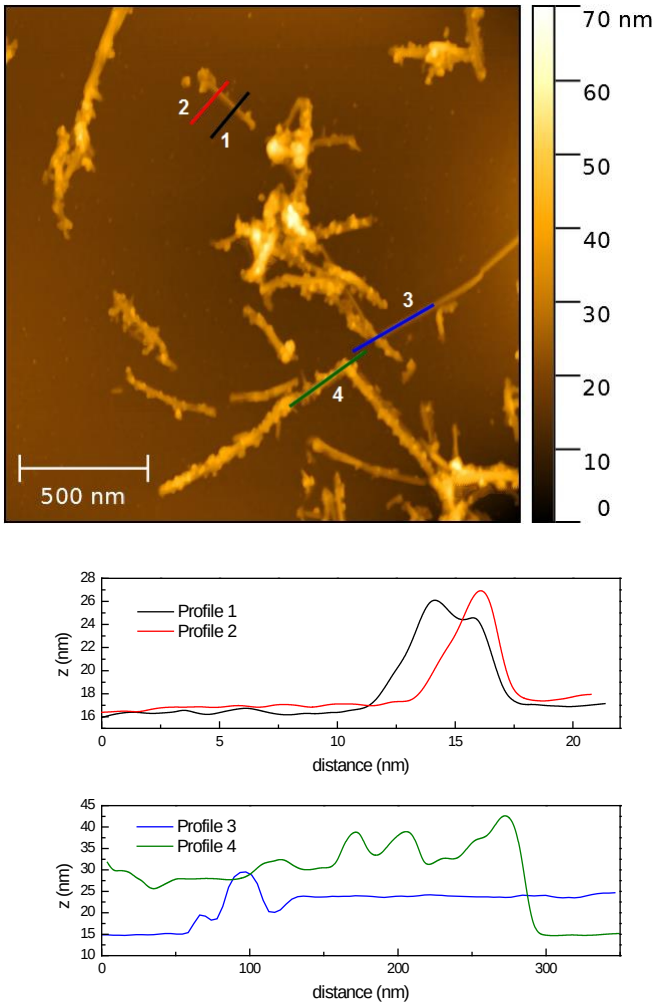


Fig. 2. AFM height image and their representative profile cross section for lysozyme fibrils doped

with magnetic nanoparticles after 1 hour incubation.

Experimental SAXS curves for the mixtures of amyloid aggregates with MNPs (Fig. 3) were analyzed by applying the Indirect Fourier Transform (IFT) method developed by Glatter [15] in the version of Pedersen [16]. Taking into account elongated shape of the particles, the cross-section pair distance distribution (PDD) functions, $p_{cs}(r)$, have been obtained in Fig.4 that indicates the helical structure of lysozyme fibrils which is similar to previous works [17,18]. The power- law type scattering at small q values (linear parts in the graph) with power exponent close to -1 indicates the presence of elongated particles. Model of cylinder-like shape is an approximation of the real shape of the amyloid aggregates.

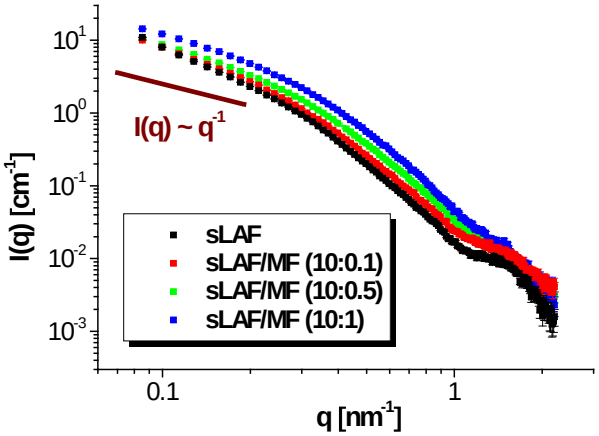


Fig. 3 Experimental SAXS curves for sLAF and mixtures of sLAF and various concentrations of MNPs.

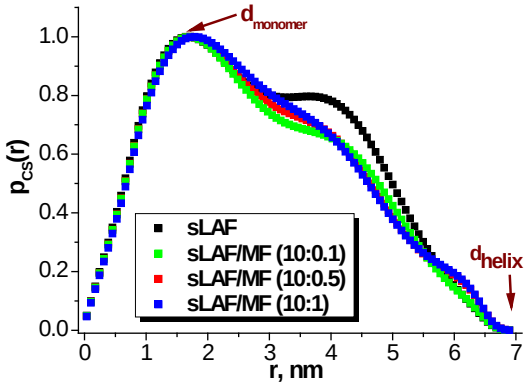


Fig. 4 The cross-section of PDD functions as a result of the IFT procedure for scattering from mixture of sLAF and various concentrations of MNPs.

Fibrils have shown some periodic structure most probably helical. Radius of primary component is~ 1.4-2 nm and diameter of whole helixes slightly less than 7 nm. By adding magnetic fluid to lysozyme fibrils, the structure of the lysozyme was changed. It leads to disappearing of second maxima (helical structure) and some shift of first maximum (primary protein component). The shift of maximum for primary component points to adsorption of MF and increasing of effective cross section (Fig. 4).

4. Conclusion

Our results showed that addition of small amount of MNPs leads to partly loosing the helical structure of amyloid fibrils. The pitch length of supposed helixes does not change significantly and we obtained less defined cross section diameter. It should be responsible for the loss of helical features of fibrils and it is first step to order such complex system applying magnetic field.

Acknowledgement

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