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On the adsorption of magnetite nanoparticles on lysozyme amyloid fibrils

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ABSTRACT

An adsorption of magnetic nanoparticles (MNP) from electrostatically stabilized aqueous ferrofluids on amyloid fibrils of hen egg white lysozyme (HEWL) in 2 mg/mL acidic dispersions have been detected for the MNP concentration range of 0.01–0.1 vol.%. The association of the MNP with amyloid fibrils has been characterized by transmission electron microscopy (TEM), small-angle X-ray scattering (SAXS) and magneto-optical measurements. It has been observed that the extent of adsorption is determined by the MNP concentration. When increasing the MNP concentration the formed aggregates of magnetic particles repeat the general rod-like structure of the fibrils. The effect is not observed when MNP are mixed with the solution of lysozyme monomers. The adsorption has been investigated with the aim to clarify previously found disaggregation activity of MNP in amyloid fibrils dispersions and to get deeper insight into interaction processes between amyloids and MNP. The observed effect is also discussed with respect to potential applications for ordering lysozyme amyloid fibrils in a liquid crystal phase under external magnetic fields.

1. Introduction

The interaction of nanoparticles with biological macromolecules and their complex associates is of current interest for applications in medicine and biotechnology. Thus, a great effort is given to the study of amyloidal aggregation of polypeptides in different conditions. Amyloid fibrils represent energetically more stable state as compared to the functional monomeric state of proteins [1,2]. The basic characteristic units of these formations are protofilaments, the polypeptide aggregates predominantly composed of cross-β-sheet structures where β-strands are arranged perpendicular to the constituent axis while the β-sheets are parallel to the axis [3]. The corresponding rod-like morphology is characterized by an aggregate diameter at the level of 10–20 nm (depending on the monomeric protein and incubation conditions) and length up to few micrometers. Protofilaments intertwine to form protofibrils, and the fibrils form when protofibrils associate laterally or twist around the main axis [4,5]. In living organisms, amyloids are associated with various serious diseases known as amyloidosis (e.g. Alzheimer's and Parkinson's diseases, Creutzfeldt–Jakob disease, type II diabetes and others) [1,6]. At the same time, amyloid fibrils being extended polymeric formations exhibiting specific properties are attractive as a basis for new functional nanomaterials [7]. A practical advantage is that they can be efficiently and easily prepared in vitro.

In this connection, several studies have demonstrated the applications of amyloids based on the "bottom-up" strategy including templates for the fabrication of conductive nanowires [8] and the coating of peptide nanotubes with silver and gold, which resulted

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in the fabrication of coaxial trilayer metal–peptide–metal nanocables [9]. Recently, a progress has been achieved in the development of new composite materials composed of various forms of carbon and amyloid fibrils. Thus, graphene-amyloid composites combining high conductivity and reversible change in shape with variation in humidity can be used as templates for biosensors for measuring enzyme activity [10], while amyloid-active carbon hybrid membranes have been utilized for water purification [11].

The formation and structural stability of protein amyloid aggregates can be influenced by nanoparticles. The copolymer particles, cerium oxide particles, carbon nanotubes and quantum dots enhance the rate of fibril formation from β_2-microglobulin by decreasing the lag time of nucleation [12]. The lag phase of fibrillization depends on the amount and nature of the particle surface. Wu et al. observed that TiO_2 nanoparticles promote amyloid-β (Aβ peptide) aggregation [13]. In contrast, the significant inhibition of Aβ amyloid polymerization was observed for fluorinated nanoparticles and hydrophobic teflon nanoparticles [14]. The obtained results suggest that nanoparticles interfere with the protein amyloid aggregation differently and the final impact depends on diverse physical and chemical properties of nanoparticles such as size, surface composition, charge and structure.

Previously, the interaction of magnetic nanoparticles (MNP) with amyloid aggregates was reported [15–17]. Namely, the inhibition and disaggregation activities of stabilized magnetite (Fe_3O_4) nanoparticles added to the lysozyme and insulin amyloid solutions were observed above some critical MNP concentration.

These findings opened a new field of research related to the utilization of the magnetic properties of MNP in the potential amyloidosis treatment. Up to date, there is no clear explanation of these effects which require detailed and systematic studies. In addition, the Fe_3O_4-based NPs possess a unique property of superparamagnetism that confers advantages to control the transition to a nematic liquid crystal phase in amyloid fibril dispersions which is of current interest [18–20]. This transition was observed for lysozyme amyloid fibrils [21]. The possibilities for orientating amyloid fibrils of β-lactoglobulin with MNP under an external magnetic field have been demonstrated by Bolisetty and co-workers [22].

The behavior of magnetic nanoparticles in the mentioned complex multicomponent dispersions is an important aspect determining the possibilities for regulating the structural organization and properties of these systems including their stability in various conditions. In the previous structural studies of bulk mixed solutions of MNP with amyloid fibrils [22] by small-angle neutron scattering the focus was investigation of the amyloid aggregates (scattering from MNP was eliminated due to contrast in heavy water). Here, the work was aimed at obtaining the structural information about the MNP spatial distribution in a model amyloid dispersion depending on the particle concentration and relating it with the corresponding changes in the magnetic characteristics of the system. For this purpose, we have followed the structural organization of magnetite MNP in the mixed dispersions with amyloid fibrils of hen egg white lysozyme (HEWL) in the MNP concentration range below their disaggregation activity [15].

Different techniques mostly sensitive to magnetite against other dispersion components have been used including transmission electron microscopy (TEM), small-angle X-ray scattering (SAXS) and magneto-optical Faraday rotation (FR). The combination of a direct imaging technique such as TEM, which provides high-resolution structure determination in real space, with high statistically averaged structural parameters from q-space obtained by SAXS and magnetic characteristics by FR allows the investigation of the extent of adsorption of MNP on the fibrils depending on the particle concentration in bulk. We have characterized the associates formed due to the interaction of MNP with HEWL amyloids in dispersions. The conclusion about specific interaction has been made basing on the comparison of the behavior of MNP in amyloid and monomer (native) lysozyme solutions.

HEWL is one of the amyloidogenic proteins often used in the study of model amyloid aggregation due to the well-established three dimensional structure, folding-unfolding mechanism, conformational stability [23–25], as well as the conditions for amyloid fibrillization in vitro. The lysozyme amyloid fibrils (LAF) can form in the presence of detergents [26,27], after incubation at low pH at 57 °C [28] or due to reduction of the protein (using a reduction agent) [29]. Recent investigations give examples of more possible complex structures of LAF with multistrand nature [30,31].

The research presented herein is a first step to clarify the interaction processes between amyloid fibrils and MNP, causing the amyloid disaggregation upon further increase in the MNP concentration. The observed changes in the MNP reorganization and their effect on the magnetic properties of the system are discussed with respect to the prospects to use MNP for regulating lysozyme fibril orientation by external magnetic fields and synthesis of ferronematics based on the revealed MNP/LAF associates.

2. Materials and methods

2.1. Chemicals

Hen egg white lysozyme (lyophilized powder, lot number L6876, ~50.000 units mg^{-1} protein), Thioflavin T (ThT), $FeCl_3{\cdot}6H_2O$ (31 232), $FeSO_4{\cdot}7H_2O$ (215 422) and NH_4OH were obtained from Sigma-Aldrich Chemical Company (St Louis, MO). All other chemicals were obtained from Sigma or Fluka and were of analytical reagent grade.

2.2. Lysozyme amyloid fibrillization

Lysozyme powder was dissolved to a final concentration of 2 mg/mL in 70 mM glycine buffer in the presence of 80 mM NaCl, pH 2.7. Spontaneous amyloid fibrillization of lysozyme was induced by means of incubation at 65 °C in a thermomixer (Eppendorf comfort) and constant stirring (1200 rpm) for 2 h [15,32]. The formation of lysozyme amyloid fibrils was monitored by Thioflavin T assay and confirmed by microscopic techniques (TEM or atomic force microscopy (AFM)).

2.3. Magnetic nanoparticles

The magnetite nanoparticles were prepared by co-precipitation of Fe^{2+} and Fe^{3+} salts by NH_4OH at 60 °C. In a typical synthesis to obtain 1 g of Fe_3O_4 precipitate, 0.86 g of $FeCl_2{\cdot}4H_2O$ and 2.35 g of $FeCl_3{\cdot}6H_2O$ were dissolved in 40 mL of deionized water by vigorous stirring, such that the molar ratio of $Fe^{3+}/Fe^{2+} = 2$. After the solution was heated up to 60 °C, 5.6 mL of 25% NH_4OH was added. 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 18.027 mg/mL [33]. The mean hydrodynamic diameter of MNP of about 25 nm was estimated by dynamic light scattering and the magnetite size of about 10 nm was found from the magnetization analysis.

2.4. Preparation of protein-magnetic nanoparticle mixtures

The magnetic fluid was added to the LAF dispersion (final LAF concentration was always kept constant and equal to 2 mg/mL) to achieve three different concentrations of MNP of 0.5, 2.5 and 5 mg/mL, respectively. Aqueous dispersions of the initial monomer (native) lysozyme (ML) and MNP were also prepared with the same