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Destroying activity of glycine coated magnetic nanoparticles on lysozyme, α-lactalbumin, insulin and α-crystallin amyloid fibrils

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ABSTRACT

A great variety of human protein deposition and protein aggregation diseases (Alzheimer's disease, diabetes mellitus, cataract, systemic amyloidosis and other) have been associated with the accumulation of amyloid fibrils in different tissues. Therefore, development of the agents able to reduce amyloid deposits represents an attractive strategy for their treatment. We have investigated ability of glycine coated magnetic nanoparticles (Gly-MNPs) to destroy protein amyloid fibrils. The properties of Gly-MNPs were characterized with the aim to identify the optimized conditions for the glycine adsorption on surface of MNPs. It was found that Gly-MNPs have superparamagnetic behavior and their size, isoelectric point and stability depend on the amount of the glycine in the samples. The obtained results suggest that optimal weight ratio (w/w) for the modification of MNPs by glycine (Gly/Fe_3O_4) is equal to 5/1. The selected Gly_5-$MNPs_1$ were used for the study of their effect on amyloid fibrils of four globular proteins, namely lysozyme, bovine α-lactalbumin, insulin and α-crystallin. It was found that Gly_5-$MNPs_1$ destroy lysozyme, α-lactalbumin and insulin amyloid fibrils in concentration dependence manner. However, Gly_5-$MNPs_1$ were not able significantly destroy bovine α-crystallin amyloid fibrils. We assume that obtained results represent important contribution for rational design of potential therapeutics of amyloid diseases based on nanoparticles.

1. Introduction

Protein amyloid fibrils are highly ordered, insoluble and very stable nanostructures associated with more than 50 amyloid diseases such as Alzheimer's and Parkinson's diseases, diabetes mellitus and others [1–4]. In these diseases the normally soluble proteins are transforming into amyloid aggregates characteristic of high content of cross β-sheet structure. The deposition of poly/peptides in amyloid form is the main cause of human organs and tissues damage [5]. It seems that under appropriate conditions every poly/peptide has propensity to self-assembly into amyloid structures; therefore, formation of amyloid fibrils is generic property of poly/peptide chain [6–8].

There are several globular proteins with high propensity to form amyloid fibrils. The amyloid aggregation of human lysozyme, a bacteriolytic enzyme presented in the liver, milk, saliva and serum, is associated with fatal hereditary systemic lysozyme amyloidosis [9,10]. Hen egg white lysozyme (HEWL) is often used to investigate the lysozyme amyloid self-assembly as it is highly homologous to human variant [11,12]. HEWL is a protein consisting of 129 amino acids (14.7 kDa) and its physico-chemical properties are well characterized [9]. The α-lactalbumin, 123 amino acids (14.17 kDa) protein, is a predominant protein component of milk and the second most abundant protein in whey [13]. α-Lactalbumin can evoke a variety of physiological effects [14] such as modulation of the lactose synthase activity or apoptotic activity in tumor cells [15,16]. Till now the self-assembly of α-lactalbumin was not associated to any specific amyloid disease. Insulin is a small globular 51 amino acids (5.8 kDa) protein hormone that is crucial for control of the glucose metabolism and diabetes treatment. Insulin amyloid deposits have been found in the sites of subcutaneous drug application in patients with long-term diabetes treatment. Insulin amyloid aggregation causes also a serious problem in the production, storage and delivery of this important biopharmaceutical compound as well as in application of the insulin pumps [17]. The α-crystallins are large complex proteins (~700 kDa) primarily found within the mammalian eye lens where they contribute to lens transparency and refractive properties [18]. Crystallin aggregation is the most important

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factor in cataract formation [19]. With ageing of the lens, α-crystallin is post-translation modified, unfolded and aggregated into amorphous and/or amyloid fibrils. Factors that lead to protein aggregation include mutations in crystallin proteins, which are known to cause congenital cataracts or oxidative stress, which in turn contributes to age related cataracts [20–22].

Since the presence of amyloid fibrillar deposits is the main hallmark of amyloid diseases, development of the agents able to combat them is one of key approaches for their treatment. In last decade, various molecules with ability to inhibit amyloid fibrillization or destroy amyloid fibrils have been found. The anti-amyloid properties were observed for small molecules, peptides, antibodies or heat shock proteins [23–25]. Recently, the nanoparticles have been suggested as one of a possible agent affecting amyloid aggregation. It is mainly due to their unique properties as high surface/volume ratio and possibility of various surface modification providing large and specific surface areas for interaction with biomacromolecules [26,27]. It was found that copolymer or cerium nanoparticles or quantum dots significantly enhance the rate of fibril formation [28]. While gold nanoparticles have been reported to induce formation of protein aggregates [29], the ablation of amyloid aggregates by gold nanoparticles has also been observed [30,31]. The anti-amyloid activities were found for fluorinated nanoparticles [32] as well as for fullerenol [33].

The magnetic properties, relative high biocompatibility and low toxicity increase application of magnetic nanoparticles (MNPs) in biomedicine as in magnetic resonance imaging, drug delivery, bioseparation and hyperthermia [34–37]. However, there are only few reports on the interaction of the magnetic nanoparticles with amyloid aggregation of poly/peptides. Mahmoudi et al. investigated controversy effect of magnetic nanoparticles on kinetic of Aβ fibrillization. While lower concentrations of superparamagnetic nanoparticles inhibited fibrillation, higher concentrations increased the rate of Aβ fibrillation [38]. Sen et al. found that $MnFe_2O_4$ nanoparticles inhibit fibrillation of human serum albumin [39]. The fluorescent MNPs were also used for detection of Aβ plaques and their removal by magnetic field [40]. Wang et al. reported that the heparin-functionalized magnetic glyconanoparticles can bind to Aβ, induce the formation of fibrils, and protect neuronal cells from Aβ induced cell death [41]. In our previous studies we have found that Fe_3O_4-based nanoparticles are able to reduce amyloid aggregation of lysozyme or insulin [42–44].

The aim of the present study was characterization of the properties of the prepared glycine (Gly) coated magnetic nanoparticles and identification of the optimized conditions for the adsorption of glycine on MNPs. It was found that Gly-MNPs have superparamagnetic behavior and their size, isoelectric point and stability depend on the amount of the glycine in samples. The obtained results suggest that optimal weight ratio (w/w) for the modification of MNPs by glycine (Gly/Fe_3O_4) was equal to 5/1. The selected Gly_5-$MNPs_1$ were used for the study of their effect on amyloid fibrils of four proteins, namely lysozyme, α-lactalbumin, insulin and α-crystallin. We have found that Gly_5-$MNPs_1$ destroy lysozyme, α-lactalbumin and insulin amyloid fibrils in concentration dependence manner. However, Gly_5-$MNPs_1$ were not able significantly destroy bovine α-crystallin amyloid fibrils. We assume that obtained results represent important contribution for rational design of potential therapeutics of amyloid diseases based on nanoparticles.

2. Experiment

2.1. Materials

Glycine (Gly) (99%), ferric chloride hexahydrate ($FeCl_3{\cdot}6H_2O$), ferrous sulfate heptahydrate ($FeSO_4{\cdot}7H_2O$), ammonium hydroxide (NH_4OH), perchloric acid ($HClO_4$), lysozyme from hen egg-white (lyophilized powder, lot number L 6876, ~40.000 units/mg protein), α-lactalbumin from bovine milk (type III, calcium depleted, purity ≥85%, L6010), insulin (human recombinant insulin, expressed in yeast, lot number I2643, ~24 IU/mg protein), α-crystallin from bovine eye lens (C4163, purity ≥70%), NaCl, glycine (Gly), HCl, trifluoroethanol (TFE) and thioflavin T (ThT) were obtained from Sigma Aldrich Chemical Company (St. Louis, MO). The protein concentrations were determined spectrophotometrically (UV VIS JASCO V-630 spectrophotometer) using an extinction coefficient measured at λ = 280 nm. Ultrapure deionized water (Milli-Q) was used throughout the experiments.

2.2. Preparation of Fe_3O_4 nanoparticles stabilized in $HClO_4$ and their functionalization with glycine

Iron oxide (Fe_3O_4) magnetic nanoparticles (MNPs) were synthesized by co-precipitation method and stabilized with perchloric acid by the method described by Antal et al. [42,45,46]. The concentration of Fe_3O_4 in prepared aqueous solution of MNPs was estimated by thiocyanate colorimetry [47] and was equal to 30 mg/mL. The functionalization of iron oxide MNPs with amino acid glycine to obtain the Gly-MNPs was carried out by mixing of glycine aqueous solution (c_{Gly} = 100 mg/mL) with nanosuspension of MNPs at different weight ratio (w/w) of Gly/Fe_3O_4 ranging from 0 to 50 during 72 h at 25 °C. The samples were centrifuged at 35,000 rpm for 20 min to separate unbound amino acid. For determination of Gly concentration in supernatants the spectrophotometric method (described below) was used. Bound amount of Gly was then calculated as difference between total and unbound amount of Gly.

2.3. UV/VIS spectroscopy

The adsorption of Gly onto the MNPs surface was quantified using the spectrophotometric method with ninhydrin using SPECORD® PC 40 UV VIS spectrophotometer [48]. To obtain the calibration curve, amino acid solutions with different volumes (0.1–0.45 mL) were put to the test tubes. Then the distilled water was added to the all tubes to make up the 4 mL volume. As a blank the distilled water was used. In the next step, 1 mL of 2% w/v ninhydrin reagent was added to all the test tubes and stirred using vortex. The tubes were placed in thermomixer for 15 min at the temperature 100 °C and subsequently cooled in cold water. After addition of 1 mL of ethanol/water (1:1) the mixtures were mixed and the absorbance at 570 nm was recorded for each sample. The same procedure was used to determine Gly concentrations in supernatants.

2.4. Nanoparticles characterization

Zetasizer NanoZS (Malvern Instruments) was used for determination of hydrodynamic size distribution of MNPs and Gly-MNPs using the dynamic light scattering method (DLS) with a scattering angle of 173° as well as for zeta potential measurements. Zeta potential is commonly used to predict and control the stability of the sample. It is proportional to electrophoretic mobility that is determined by Laser Doppler Velocimetry.

Scanning electron microscopy (SEM, JEOL 7000F microscope) was used for characterization of the morphology of both MNPs and Gly-MNPs. The samples were prepared by deposition of the sample containing MNPs or Gly-MNPs on a double sided carbon tape stuck on the SEM sample stub and dried under vacuum prior to sputtering with carbon and subsequent observation.

The field dependent magnetization measurements were carried out using Magnetic Property Measuring System model MPMS-XL-5 (Quantum Design) equipped with 5 T superconducting magnet.

2.5. Formation of protein amyloid fibrils

Amyloid fibrillization of lysozyme (LF) – Lysozyme was dissolved to a final concentration of 5 mg/mL in 70 mM glycine buffer containing