FLUORESCENCE STUDY OF THE EFFECT OF SILVER NANOPARTICLES ON INSULIN AMYLOID FIBRIL FORMATION

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Silver nanoparticles (AgNPs) have been the most thoroughly investigated nanomaterials over the past decades due to its high antibacterial, antifungal and catalytic activities. AgNPs have been applied in water purification systems, in the food and clothing industries, in biomedicine, e.g. as drugs against antibiotic resistant bacteria, for DNA/RNA detection via specific probes, etc. Chemical and optical properties of AgNPs are tunable, depending on size and shape of nanosilver, which are determined by the method of synthesis. In turn, using AgNPs as biological agents desires application of the principles of "green chemistry", leading to an eco-friendly and cheap fabrication process. Among the nanofactories capable of forming "ecology clean" AgNPs, fungi are better that plants and bacteria due to the easier large-scale production of nanosilver. Despite a huge number of AgNPs have been formed using fungi, further investigations are needed to establish a correlation between the method of synthesis (including type of fungi, concentration of fungi and $AgNO_3$, temperature, purification conditions) and the physico-chemical properties of nanosilver, that is necessary for the functionalization of AgNPs. One of the important and poorly studied area of potential applications of AgNPs is inhibition of amyloid fibril formation that is the early marker of the development of the severe human disorders, including Alzheimer's disease, Parkinson disease, systemic amyloidosis, etc. Amyloid fibrils are highly ordered insoluble protein aggregates, deposited in a variety of organs and tissues in over 20 human diseases. The present study was aimed at testing the potential of the novel AgNPs, referred to here as QD1, QD2, QD3, QD4, QD5, QD6, QD7 and QD8, which were synthesized extracellulary by reduction of Ag+ in the genus Pleurotus fungi aqueous extracts, to inhibit insulin amyloid fibril formation in vitro. The X-ray diffraction and TEM analysis showed that most of the AgNPs were ~10 nm in diameter, while dynamic light scattering and zeta-potential measurements suggested the aggregation stability of these colloidal systems. Absorption peaks of the AgNPs were within the range of 406–430 nm, although the nanoparticles showed weak fluorescence intensities at ~590–630 nm both in the absence and in the presence of insulin amyloid fibrils (InsF). Next, the kinetics of InsF amyloid formation (at 52 °C, pH 7.4, under linear shaking) was monitored using the Thioflavin T (ThT) assay, revealing that at low concentration of the AgNPs, corresponding to the absorbance values of 0.006 and 0.03, the ThT fluorescence intensity at 480 nm reached a plateau after 18 h. Furthermore, the final ThT intensity values were ~2 orders of magnitude higher than those before protein incubation, suggesting the absence of any inhibition effect of the AgNPs on the insulin fibrillization. In turn, at higher absorbance values of the AgNPs (0.1–0.5), the final ThT fluorescence intensity was 2–3 times weaker, as compared to that in the control sample (in the absence of the AgNPs). Furthermore, presence of QD2 in the insulin sample incubated at 37 °C, pH 7.4, under linear shaking, led to the ~1.3-fold increase in the lag time of the fibril formation, suggesting that insulin interactions with QD2 (most likely, with the QD2 capping proteins) induced retardation of the amyloid nucleation process. Overall, our study, revealing the ability of the AgNPs to inhibit insulin amyloid fibrillization in vitro, can be useful for the development of the anti-amyloid drugs based of the AgNPs.