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Presence of Magnetic Fluids Leads to the Inhibition of Insulin Amyloid Aggregation

Z. Gazova[a,*], K. Siposova[a,b], M. Koneracka[a], A. Antosova[a], V. Zavisova[a], M. Kubovcikova[a], D. Fedunova[a], J. Bagelova[a], N. Tomasovicova[a], Z. Daxnerova[c] and P. Kopcansky[a]

[a]Institute of Experimental Physics, Slovak Academy of Sciences, Kosice, Slovakia

[b]Department of Biochemistry, Faculty of Science, Safarik University, Kosice, Slovakia

[c]Institute of Biology and Ecology, Faculty of Science, Safarik University, Kosice, Slovakia

Insulin amyloid aggregation caused serious problems in the treatment of diabetes by insulin injection or by insulin pumps. *In vitro* formation of insulin amyloid fibrils was investigated in presence of several types of magnetic fluids. Interaction of magnetic fluids with insulin amyloid aggregates led to decrease of insulin fibrillization. The inhibiting activities are affected by coating layer of studied magnetic fluids as well as by their physical properties (diameter, concentration of magnetic particles). The highest inhibiting efficiencies were detected for sterically stabilized magnetic fluids in saline solution (75%) and for magnetic fluids modified by dextran (80%).

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1. Introduction

Amyloid aggregation of proteins is related to incurable human amyloidosis, such as Alzheimer's and Huntington's diseases, prion-related diseases and diabetes type II. The common feature of amyloid diseases is presence of amyloid deposits in various parts of body consisting mainly of aggregated poly/peptide typical for given disease [1]. The precise mechanisms by which the normally soluble proteins forms amyloid aggregates (oligomers, pores, ordered fibrils) is unknown, however, it is generally accepted that presence of amyloid deposits in different tissues has toxic consequence to various cell types leading to their dysfunction or death [2].

In the past few years the inhibiting activity was detected for heat shock proteins, short peptides, antibodies, surfactants and small molecules [3, 4]. Several types of nanoparticles were identified as intensive inhibitors of amyloid aggregation. Recently, we have found that Fe_3O_4 based magnetic nanoparticles prevent lysozyme amyloid fibrillization [5].

We concern our study to investigate the ability of four magnetic fluids (MFs) to affect the insulin amyloid aggregation. We have found that studied magnetic fluids inhibit insulin polymerization differently in dependence on MF's properties.

2. Experimental

The magnetic particles were prepared by co-precipitation using ferric and ferrous salts in ammonium hydroxide. The magnetic nanoparticles were stabilized electrostatically with perchloric acid (MF1) or sterically with sodium oleate (MF2–MF4) and dispersed in water (MF1, MF2 and MF3) or in physiological saline solution (MF4). Magnetic nanoparticles were functionalized by bovine serum albumin (BSA) (MF2) or dextran (MF3), which were added during stirring and then heating up to 50 °C in the w/w ratio BSA/Fe_3O_4 = 0.35 and dextran/Fe_3O_4 = 0.5, respectively.

Hydrodynamic diameter of the prepared magnetic fluids, considering the magnetic core and the coating layer of BSA or dextran were determined by dynamic light scattering (DLS) using a Zetasizer Nano-ZS from Malvern Instrument. To characterize the morphology and microstructure of magnetic fluids the scanning electron microscopy (SEM, JEOL 7000F microscope) was used.

Insulin amyloid fibrils were prepared by incubation (120 min) of 58 μg/ml human insulin (10 μM) at 65 °C under constant stirring in 100 mM NaCl, pH 1.6 (for MF1 and MF2) and in 50 mM phosphate buffer solution, pH 7.5 (for MF3 and MF4). The formation of insulin aggregates was monitored by Thioflavin (ThT) fluorescence assay and by transmission electron microscopy (TEM).

ThT assay is sensitive to the binding of ThT to amyloid fibrils accompanied with significant enhancement of ThT fluorescence which is not observed for native protein. Fluorescence intensity was measured by spectrofluorimeter Shimadzu RF-5000. The excitation was set at 440 nm and the emission recorded at 485 nm. The TEM images were obtained by Tesla BS 500 operating at 60 kV.

The effect of MFs on insulin fibrillization was investigated by adding the magnetic fluid to the soluble insulin (10 μM) before starting the process of amyloid aggregation (described above). The influence of MFs on the

* corresponding author; e-mail: gazova@saske.sk

insulin polymerization was observed at two w/w ratios of insulin (Ins) and magnetite component of MF, namely for ratios Ins:MF = 1:1 and 1:2. As a control, the protein was replaced with water to measure the fluorescence of the nanoparticles.

3. Results and discussion

As a first step of our study the basic characteristics of magnetic fluids MF1–MF4 were established. The average core diameter $D_{\mathrm{TEM}} = 10$ nm was determined by TEM. SEM image (Fig. 1, left, MF3 was chosen as representative image) indicates that particles in MFs have nearly spherical shape and the surface is primarily smooth. The hydrodynamic diameters D_H of studied MFs were observed by DLS technique and data are summarized in Table. The adsorbed layer thickness was estimated from the difference in size between naked and covered (with BSA (MF2) and dextran (MF3)) magnetic fluid. The BSA and dextran coating thickness were found to be about 13 nm and 10 nm, respectively. The obtained MF hydrodynamic diameters suggest that all studied magnetic fluids fit the requirements for intravenous application (hydrodynamic diameter less than 150 nm).

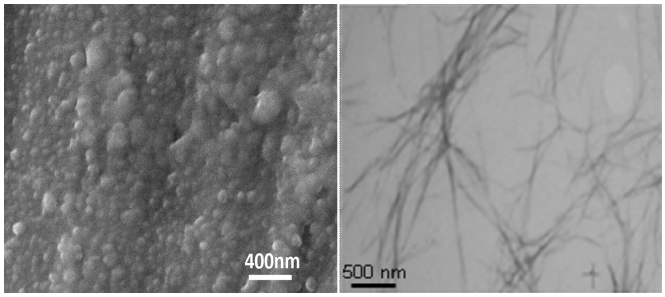

Fig. 1. SEM image of nanoparticles constituting magnetic fluid MF3 (left) and TEM image of insulin amyloid fibrils (right).

TABLE

Hydrodynamic diameter D_H, saturation magnetization M_{s} and concentration of magnetic particles $c_{\mathrm{Fe_3O_4}}$.

Sample	D_H [nm] DLS	M_{s} [mT]	$c_{\mathrm{Fe_3O_4}}$ [mg/ml]
MF1	26	1.3	36
MF2	63	6.3	85
MF3	65	7.2	90
MF4	80	13.7	175

The magnetic properties of prepared MFs were characterized by SQUID magnetometer. The all samples exhibited a typical superparamagnetic behaviour without a hysteresis at room temperature (data not shown). The saturation magnetizations (M_{s}) of samples and magnetic particle concentrations ($c_{\mathrm{Fe_3O_4}}$) calculated from magnetic measurements are given in Table.

The human insulin was chosen as amyloidogenic protein, whose aggregation caused serious problems in the treatment of diabetes by insulin injection or by insulin pumps. Typical amyloid character of insulin aggregates was observed by ThT fluorescence assay as increase of fluorescence intensity (data not shown) and confirmed by TEM (Fig. 1, right).

We have studied formation of insulin amyloid aggregates I_{agg} in the presence of magnetic fluid at two w/w ratio of insulin (Ins) and magnetite component of MF (Ins:MF = 1:1 and 1:2) by ThT assay where lowering of fluorescence values indicates decrease of insulin amyloid aggregation, i.e. inhibiting activity of MF. The fluorescence intensities observed for MF alone or in the presence of ThT were negligible. Interaction of MF with insulin amyloid aggregation led to decrease of insulin fibrillization; however the extent of inhibiting activity was different as it is shown in Fig. 2 (the observed fluorescence intensities were normalized to the fluorescence signal detected for the insulin amyloid aggregates alone (I_{agg})). For the ratio Ins:MF = 1:1 the MF1 and MF2 inhibit polymerization not very intensively, about 45% and 20% inhibiting activities were observed. The inhibition of about 70% was detected for both the MF3 and the MF4.

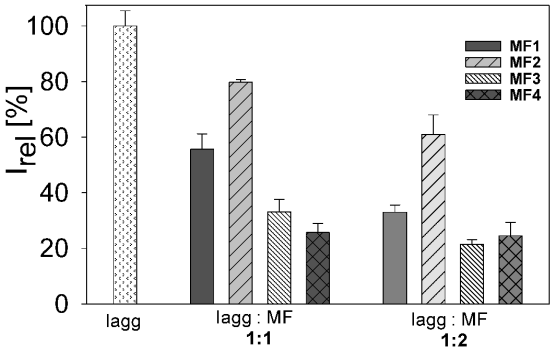

Fig. 2. Inhibition of insulin amyloid fibrillization by magnetic fluids MF1–MF4 observed by ThT assay. The fluorescence intensity signal was normalized to the signal detected for insulin amyloid aggregates formed without MFs (I_{agg}).

For the ratio Ins:MF = 1:2 the trend of the MFs to affect insulin aggregation is similar to that observed for previous ratio. However, the efficiencies of inhibition are higher. The magnetic fluid MF2 prevents formation of aggregates less effectively (40% inhibition). In presence of MF1 there is about 65% decrease of the amount of insulin aggregates in comparison to untreated insulin fibrillization. The most effective inhibiting activities of about 80% and 75% were observed for magnetic fluids MF3 and MF4.

It can be concluded that interaction of MF with insulin amyloid aggregates led to decrease of insulin fibrillization, the best inhibiting activities were detected for

MF3 and MF4, respectively. We suggest that efficiency of inhibitors depends on the physical parameters of studied MFs together with properties of the coating layer. This assumption is supported by the results observed for inhibiting abilities of MF2 and MF3. The physical parameters of MF2 and MF3 are nearly identical, however, the MF3 inhibits more effectively. We suggest that it is due to different properties of the coating layer. Interaction of the polysaccharide shell (in MF3) with insulin aggregates leads to higher inhibiting activity compared to protein modification in MF2.

4. Conclusion

The presence of magnetic fluids (MF1–MF4) caused inhibition of insulin amyloid aggregation. Results indicate that magnetite component of magnetic fluids is important for inhibiting activity; however, the MF physical properties together with character of NP coating determine the extent of inhibition. The most significant inhibiting activity was observed for two MFs characteristic magnetic nanoparticles stabilized by sodium oleate and dispersed in physiological saline solution (MF4) or in water and functionalized with dextran (MF3). We assume that present findings make effective magnetic fluids of potential interest for solution of the problems associated with insulin amyloid aggregation.

Acknowledgments

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