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Characterization of Fe_3O_4 Magnetic Nanoparticles Modified with Dextran and Investigation of Their Interaction with Protein Amyloid Aggregates

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Functionalised magnetic nanoparticles composed of Fe_3O_4 particles stabilised by sodium oleate and subsequently modified with dextran (MFDEX) were prepared by the co-precipitation method. Their morphology and particle size distribution were observed by scanning electron microscopy and photon cross correlation spectroscopy. In order to confirm the modification of magnetite surface with dextran physical techniques, including infrared spectroscopy, thermal analysis, and magnetic measurement, were used. Finally, the effect of MFDEX on amyloid fibrillar aggregates of human insulin and hen egg white lysozyme, typical amyloidogenic proteins, was investigated. *In vitro* interaction of MFDEX with protein amyloid fibrils resulted into destruction of amyloid aggregates. The anti-amyloid activity makes MFDEX of potential interest as therapeutic agent against amyloid-related diseases.

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1. Introduction

Magnetic fluids are stable colloid suspensions of magnetic particles dispersed in various carrier liquids. Due to their special characteristics, they have gradually gained importance in numerous biological fields. Recently it has been found that Fe_3O_4 magnetic nanoparticles (MNPs) are able to interact with lysozyme amyloid aggregates [1].

The protein self-assembly into specific amyloid aggregates present in tissue is a pathological feature of many incurable human diseases such as Alzheimer's and prion diseases or diabetes type II. Lysozyme forms massive amyloid deposits in the liver and kidney of individuals affected by lysozyme hereditary systemic amyloidosis. Amyloid deposits of insulin have been observed in patients with diabetes after repeated insulin injection in the site of subcutaneous application [2].

In this paper, an oleate-stabilised dextran-modified magnetic fluid (MFDEX) has been characterized. Moreover, *in vitro* ability of MFDEX to affect the amyloid aggregates formed from human insulin or hen egg white lysozyme has been investigated.

2. Experiment and results

The magnetic particles were prepared by co-precipitation using ferric and ferrous salts and ammonium hydroxide [3]. The freshly prepared MNPs were sterically stabilized by sodium oleate (*ca.* 1:1 to Fe_3O_4) to prevent their agglomeration. By centrifuging 30 min at 9000 rpm an initial magnetic fluid (MFi) arose. Then, dextran (approximately MW = 64000) was added to achieve a dextran:Fe_3O_4 weight ratio equal to 0.5. The morphology and size of the prepared samples were observed by scanning electron microscopy (SEM) and photon cross correlation spectroscopy (PCCS). SEM analysis of the MFDEX showed almost spherical shape of particles with an average particle size about $D = 71$ nm (Fig. 1a). The particle size estimated by SEM was in good agreement with the results obtained from PCCS ($D_{\mathrm{HYDR}} = 65$ nm, Fig. 1b).

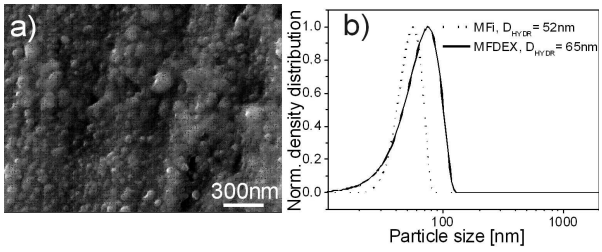

Fig. 1. SEM image of MFDEX (a), particle size distributions of MFi and MFDEX from PCCS (b).

In order to verify the coating of the magnetic particle surface by dextran, thermal analysis (DTG) and differ-

ential scanning calorimetry (DSC) for pure dextran, and lyophilised MFi and MFDEX were carried out (Fig. 2). In the case of pure dextran, the first decomposition step starting at *ca.* 50 °C was ascribed to endothermic water evolving [4]. At temperatures higher than 250 °C, the breakdown of the organic skeleton took place. For MFDEX, compared to the decomposition of pure dextran, the coated magnetite nanoparticles catalyzed the thermal decomposition of dextran (an onset of breakdown *ca.* 50 °C lower). A catalytic effect of magnetite towards the degradation of organic coating has already been reported in the literature [5].

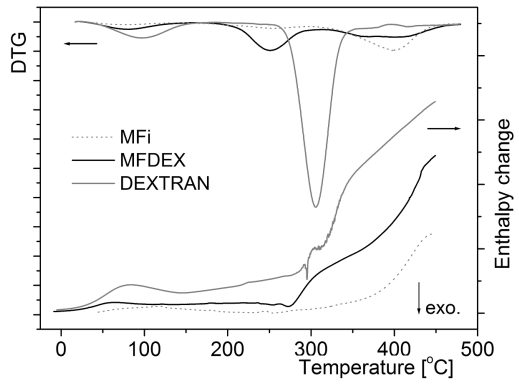

Fig. 2. DTG and DSC curves of MFi, MFDEX, and pure dextran (heating rate 10 °C min^{-1}, nitrogen atmosphere).

Attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectroscopy was applied to show the presence of the building compounds of MFDEX and to investigate the interaction between them. For the liquid samples of MFi, MFDEX, and dextran solution, difference spectra were produced by subtracting water with factor 0.99. The absorbance minimum at *ca.* 3240 cm^{-1} of MFDEX spectrum (Fig. 3) was different in comparison with spectrum of MFi. This difference above 3000 cm^{-1} corresponded with the presence of dextran molecules, as it was caused by stretching O–H vibration of polysaccharide hydroxyl groups. C–H stretching vibrations of oleate molecules were dominating in the region 3000–2800 cm^{-1}. Obvious dextran bands were at 1157 cm^{-1} (C–O stretching), 1045 cm^{-1}, and 1018 cm^{-1}. The latter two bands were related to more and less ordered structures, respectively [6]. As a shoulder of MFDEX emerged compared to the spectrum of dextran in water at 1028 cm^{-1} it could be speculated that conformational changes of dextran molecules took place during the preparation of MFDEX. Taking into account the shift of O–H stretching vibration from a band maximum of *ca.* 3330 cm^{-1} in solid dextran to *ca.* 3300 cm^{-1} in lyophilised MFDEX (spectra not shown) the interaction between dextran molecules and magnetic particles could have a form of hydrogen bonding between the hydroxyl groups of dextran and carboxylate groups of oleate anions in the outer part of the bilayer coating. In addition, the broad band around 550 cm^{-1} corresponded to Fe–O stretching.

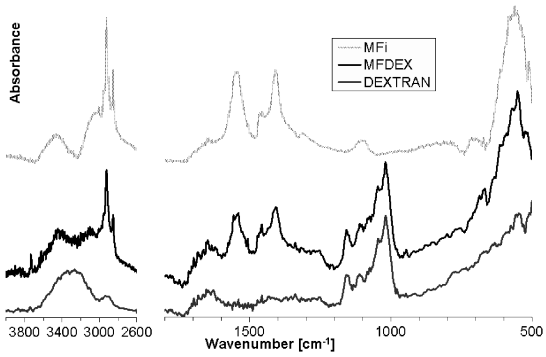

Fig. 3. ATR-FTIR difference spectra. Dextran in deionised water: 0.015 g/ml. 32 scans/sample, resolution 4 cm^{-1}, 37 °C, diamond internal reflection element. Spectra vertically shifted. Some spectral regions smoothed.

SQUID measurements of lyophilized samples MFi and MFDEX showed superparamagnetic behaviour without a hysteresis loop at room temperature and the saturation magnetizations were 15.1 and 11.5 A m^2/kg, recalculated to the mass of magnetite, respectively. The saturation magnetization of MFDEX was lower than of MFi, due to the polymeric dextran coating of the magnetic nanoparticles [7].

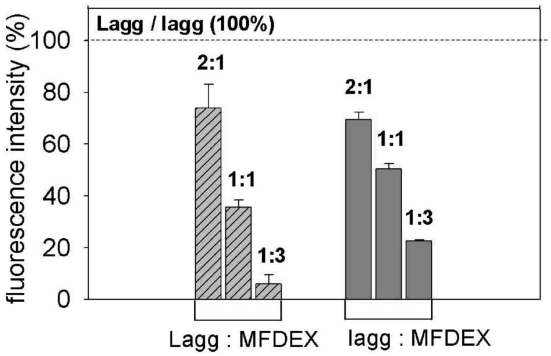

Fig. 4. Maximum fluorescence intensities detected for lysozyme amyloid fibrils L_{agg} (10 μM = 0.147 mg/ml) and insulin amyloid fibrils I_{agg} (10 μM = 0.58 mg/ml) after 24 h incubation with MFDEX observed by ThT assay.

The interaction of MFDEX with lysozyme or insulin amyloid fibrillar aggregates (L_{agg} and I_{agg}, respectively) was observed by two independent spectroscopic methods — thioflavin T (ThT) assay and 8-anilinonaphthalene-1-sulfonic acid (ANS) assay [8]. Both techniques allow quantification of the amount of the protein in the form of amyloid fibrillar aggregates as the extent of aggregation is proportional to the dye fluorescence intensity. The incubation of amyloid fibrils with MFDEX at various weight ratios (L_{agg} or I_{agg}:magnetite content of MFDEX = 2:1;

1:1; 1:3) caused a decrease of ThT fluorescence intensity indicating the reduction of the amyloid structures. The corresponding maximum fluorescence intensities detected after treatment of L_{agg} or I_{agg} with MFDEX observed by ThT assay are shown in Fig. 4 (the data are normalized to the fluorescence intensity detected for the protein amyloid aggregates alone, i.e. L_{agg} or I_{agg}). The decrease of the amount of lysozyme amyloids caused by the presence of MFDEX was *ca.* 25% at weight ratio protein amyloid:MFDEX equal to 2:1 and *ca.* 95% at ratio 1:3. A similar destruction was observed for insulin amyloid fibrils: about 30% and 80% decrease of amyloid amount were detected for ratios 2:1 and 1:3. Data obtained by ANS assay indicated similar reduction of protein amyloid aggregates (data not shown).

3. Conclusion

A dextran containing oleate stabilized superparamagnetic magnetic fluid (MFDEX) was prepared and characterized. The obtained results suggest that the presence of MFDEX led to depolymerization of both lysozyme and insulin amyloid fibrillar aggregates, which makes MFDEX of potential interest as therapeutic agent against amyloid-related diseases.

Acknowledgments

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