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Examining the effects of dextran-based polymer-coated nanoparticles on amyloid fibrillogenesis of human insulin

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ABSTRACT

More than thirty human proteins and/or peptides can aggregate to form amyloid deposits that are linked to several amyloid diseases including clinical syndrome injection-localized amyloidosis, which is correlated with the aggregation of the 51-residue polypeptide insulin. While no cure is currently available toward tackling amyloid diseases, prevention or suppression of amyloid fibrillization is considered as the primary therapeutic strategy. Nanomaterials have been demonstrated to possess great potential in the fields of biomedical diagnosis and drug delivery, they are also able to affect the amyloid aggregation of proteins. This work explores the effects of three different magnetic nanoparticles coated with dextran-based polymers on the in vitro amyloid fibrillogenesis of human insulin. Surface modification of nanoparticles with dextran-based polymers was used to improve the biocompatibility of maghemite nanoparticles. We demonstrated that insulin fibrillization may be mitigated by the studied nanoparticles in a concentration-dependent fashion as verified by ThT binding assay and transmission electron microscopy. The extent of inhibitory activity against human insulin fibril formation was found to be associated with the physico-chemical properties of nanoparticles, with the highest inhibitory activity observed for diethylaminoethyl-dextran-coated nanoparticles. Using circular dichroism spectroscopy, ANS fluorescence spectroscopy, and right-angle light scattering, we probed the structural/conformational changes and investigated the aggregating behavior of insulin upon treatment with nanoparticles. This work demonstrates that nanoparticles with an appropriate surface modification can be utilized to suppress or even inhibit amyloid fibril formation of proteins.

1. Introduction

Protein aggregation arises from a similar mechanism whereby the folded proteins alter conformation resulting in partially unfolded intermediates that eventually lead to the generation of either amorphous aggregates with no long-range structure or amyloid fibrils with ordered conformation [1]. In addition, protein aggregation has been regarded as a common feature of an array of distinct neuropathic or non-neuropathic degenerative diseases [2]. Among these are type II diabetes, Alzheimer's and Parkinson's diseases [3–5]. It has also been suggested that the precursor proteins associated with these diseases follow similar amyloid fibril formation pathways due to the morphological similarities between the amyloid fibrils derived from different proteins [3,4,6]. Amyloid fibrils have been recognized to exhibit an unbranched filamentous morphology with ~10 nm in diameter as well as ~ µm in length which are rich in cross-β secondary structure with characteristic of X-ray diffraction pattern and diagnostic dye binding. Amyloid fibril formation can be described as a stepwise process via, nucleation, oligomerization and fibril elongation [7,8]. Amyloid fibril-forming propensity of proteins is believed to be dependent upon the internal/structural factors (hydrophobicity, β-sheet content) and external/environmental factors (temperature, presence of salt, metal ion concentration, type of solvent, and pH and ionic strength of solution) [4,5].

Insulin, a 51-residue protein hormone with 21-residue A-chain and

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30-residue B-chain, regulates glucose metabolism and is used to treat diabetes. Structurally, native insulin adopts α-helix-rich conformation [9], existing as a mixture of hexamers, dimersand monomers in a solution and the proportion of which strongly depends on the environmental conditions [9,10]. Evidence has indicated that insulin exhibits an in vitro amyloid fibril-forming propensity under certain destabilizing conditions, e.g., low pH, increased ionic strength, elevated temperature and agitation [11]. Formation of insulin fibrils is often encountered at the injection site(s) of subcutaneous drug application in patients with prolonged diabetes treatment. Therefore, human insulin has been identified to be correlated with the clinical syndrome called injection-localized amyloidosis [12,13]. In addition, long-term storage and long-distance shipping of insulin would result in the generation of undesirable fibrils [14,15].

Unfortunately, there is currently no cure available for the treatment of amyloid-related diseases. There have been studies pointing to the connection between the amyloid oligomer and fibril species derived from amyloid proteins and disease pathology; thus, inhibiting the production of pathogenic fibrillar conformers or capturing these species has been viewed as the primary therapeutic strategy for the amyloid diseases. Reports have demonstrated that amyloid fibrillogenesis in vitro or in vivo can be retarded and/or inhibited by numerous molecules/substances, such as antibodies, synthetic peptides, polypeptides, heat shock proteins, small stress molecules, food additives, reducing agents, and nanoparticles [16–22]. However, it should be noted that several problems, such as poor blood–brain barrier (BBB) permeability [23,24], complex synthesis route, low in vivo stability, cytotoxicity, and low efficacy of the proposed inhibitory compounds, need to be resolved before their application as the potent anti-amyloidogenic agents within the human body [25].

Nanomaterials, with the structures with at least one spatial dimension in the range of ∼1–100 nm, have recently drawn great attention in the fields of biomedical diagnosis and drug delivery [26]. Given their unique features and/or properties including rich surface chemistry and surface functionality, enormous surface-to-volume ratio, small size, and ease of synthesis, and good in vivo stability, nanoparticles have been utilized to perturb the aggregation and/or fibrillogenesis pathway of proteins/peptides. Evidence has indicated that nanoparticles may promote, suppress, or delay the kinetics of amyloid fibril formation [27–30].

Here, attempts were made to explore how the formation of insulin amyloid fibrils was influenced by the maghemite nanoparticles that were coated with three different dextran-based polymers (e.g., dextran (Dex), carboxymethyl-dextran (CM-Dex), and diethylaminoethyl-dextran (DEAE-Dex), with the aim to develop a possible functionalization strategy of nanoparticles for efficient amyloid suppression and/or inhibition. Using a variety of spectroscopic techniques and transmission electron microscopy (TEM), we found that these dextran-based polymer-coated nanoparticles dose-dependently suppressed the amyloid fibrillogenesis of human insulin. Moreover, our results indicated that DEAE-Dex-coated nanoparticles exhibited the best inhibition activity on insulin amyloid fibril formation, suggesting that the physicochemical properties of dextrans, namely the size of nanoparticles which is dependent upon the type of coating, influence the inhibitory activity of nanoparticles. We believe the results reported here could have implications for the understanding of the role of nanomaterials/nanoparticles in amyloid fibril formation. Moreover, we assume that the presented results represent a starting point for the application of the most active dextran-coated nanoparticles as the therapeutic agents targeting insulin association/aggregation.

2. Materials and methods

2.1. Materials

Human insulin, purchased from Sigma (USA), was refrigerated upon receipt and used without further purification. Hydrochloric acid, sodium chloride, and potassium chloride were purchased from Nacalai Tesque, Inc (Japan). Glycine and sodium dodecyl sulfate was purchased from BioBasic, Inc (Canada). Dextran-based polymer coated nanoparticles, e.g., magnetic nanoparticles coated with carboxymethyl-dextran sodium salt (CM-Dex), dextran (Dex), and diethylaminoethyl-dextran (DEAE-Dex). All other chemicals, unless otherwise specified, were purchased from Sigma (USA).

2.2. Preparation of maghemite nanoparticles

Dextran-based polymer-coated magnetic nanoparticles consisting of Fe_2O_3 (maghemite) core coated with carboxymethyl-dextran (CM-Dex NPs), dextran (Dex NPs) and diethylaminoethyl-dextran (DEAE-Dex NPs) were provided by Dr. Silvio Dutz (Institute of Biomedical Engineering and Informatics, Technische Universität Ilmenau, Germany). The aforesaid maghemite nanoparticles were prepared using the Molday procedure [20] with a slight modification. Briefly, 19 g of dextran was dissolved in 75 mL of water while 5 g of $FeCl_3{\cdot}6H_2O$ and 2.1 g of $FeCl_2{\cdot}4H_2O$ were dissolved in 13 mL of 2 M HCl. Both solutions were mixed together and put to water bath (60 °C). Then, under stirring conditions, 75 mL of 7.5% ammonium hydroxide was added dropwise and the solution was mixed for another 15 min at 60 °C. The next day the ferro fluid was centrifuged (5000 rpm, 45 min; Universal 320, Hettich Zentrifugen, Germany).

2.3. In vitro insulin amyloid fibril formation

Lyophilized human insulin powder and various amounts of nanoparticles were dissolved in the glycine buffer (pH 2.0; 100 mM glycine, 100 mM NaCl, and 1.54 mM NaN_3) to prepare 0.5 mg/mL insulin samples with various mass ratios of insulin to nanoparticles at 1:0, 1:1, 1:4 and 1:5. Next, the insulin sample solutions were incubated for 3 days at 55 °C with a constant stirring at 470 rpm.

2.4. Thioflavin T (ThT) binding assay

Thioflavin T (ThT) dye can specifically bind to the cross β-sheet structure contained in amyloid fibrils and a significant increase in fluorescence intensity is detected upon binding. An extinction coefficient 26,600 $M^{-1} cm^{-1}$ at 416 nm was used to determine the ThT concentration. The stock concentration of ThT solution was 1.57 mM, which was prepared by dissolving adequate amount of ThT dye in ethanol. The stock solution was further diluted to produce the ThT solution at 20 μM. Aliquots of 12 μL insulin sample solutions (with the insulin to nanoparticles mass ratios at 1:0, 1:1, 1:4, and 1:5) taken at different time points (0, 3, 7, 24, 48, and 72 h) were mixed with 288 μL of 20 μM ThT stock solution. The final concentrations of insulin and ThT were 3.44 μM and 19.2 μM, respectivley. ThT fluorescence emission intensities of the resultant mixtures were measured on a Cary Eclipse 300 fluorescence spectrophotometer (Varian, USA) using 1-cm light path quartz cuvettes. The excitation and emission wavelengths used were 440 nm and 480 nm, respectively, and both slits were set at 5 nm. The photomultiplier tube voltage was 700 V.

2.5. Far-UV circular dichroism spectroscopy

Far-UV circular dichroism (CD) spectra was measured on a J-815 CD spectrometer (JASCO, Japan) with a 2-mm path length quartz cuvette at a scanning speed of 100 nm/min in the wavelength range of 190–260 nm. Each sample, including the insulin control and the sample with insulin and nanoparticle mass ratio at 1:1, 1:4, or 1:5, was first diluted forty times with deionized water, and then subjected to the CD measurement.

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