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Negatively charged hydrophobic nanoparticles inhibit amyloid β-protein fibrillation: The presence of an optimal charge density

Hongchen Liu [a], Baolong Xie [a], Xiaoyan Dong [a], Lei Zhang [a], Yongjian Wang [b], Fufeng Liu [a], Yan Sun [a,*]

[a] Department of Biochemical Engineering and Key Laboratory of Systems Bioengineering of the Ministry of Education, School of Chemical Engineering and Technology, Tianjin University, Tianjin 300072, China

[b] Key Laboratory of Bioactive Materials (Ministry of Education), College of Life Sciences, Nankai University, Tianjin 300071, China

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ABSTRACT

Self-assembly of amyloid β-protein (Aβ) is closely related to the pathogenesis of Alzheimer's disease (AD). Many studies suggest that polymeric nanoparticles (NPs) can inhibit Aβ fibrillogenesis depending on their electrostatic and hydrophobic properties, but the underlying molecular mechanism remains unknown. Herein, the inhibitory effect of NPs with equivalent content of hydrophobic groups but different surface negative charge densities on Aβ fibrillogenesis is examined. Firstly, the polymeric NPs of similar sizes were synthesized by copolymerizing equal proportion of *N*-isopropylacrylamide and different proportion of N-*t*-butylacrylamide and acrylic acid. Then, the inhibitory effects of these NPs on $A\beta_{42}$ fibrillization and the corresponding cytotoxicity were investigated using thioflavin T fluorescent assay, transmission electron microscopy, dynamic light scattering analysis, and cell viability assay. It was found that these NPs showed remarkable inhibitory capability against $A\beta_{42}$ fibrillogenesis and alleviated its cytotoxicity. The inhibitory capability significantly depended on the capacity of the negative surface charges carried by NPs with an increase-decrease trend. The best inhibitory efficiency was obtained at an optimal surface negative charge density. Based on the findings, a mechanistic model was proposed by considering the two interactions between $A\beta_{42}$ and NPs, namely, hydrophobic binding and electrostatic repulsion. The model suggested that at an appropriate negative charge capacity, the two opposite forces could be well-balanced, and thus led to the stretching of $A\beta_{42}$ molecules instead of the formation of a harmful β-sheet structure. The polymeric NPs of well-designed surface of proper hydrophobicity and negative charge density could thus significantly slow down the $A\beta_{42}$ fibrillation and/or result in an off-pathway aggregation with reduced cytotoxicity.

1. Introduction

Alzheimer's disease (AD), the most common form of dementia, is a chronic neurodegenerative disorder with symptoms of memory loss, disordered cognitive function, and loss of motivation [1,2]. AD is characterized by the intraneuronal neurofibrillary tangles and extracellular senile plaques in the patients' brains [3–5]. The dominant component in the senile plaque is the amyloid β-protein (Aβ), which is formed via proteolytic cleavage of the amyloid precursor protein by both β- and γ-secretases. $A\beta_{40}$ and $A\beta_{42}$ are the most prevalent isoforms and $A\beta_{42}$ is most likely to be the major toxic agents causing neuronal cell death [6–9]. Mounting evidence suggests that a key event in AD pathogenesis is the conversion of Aβ from its soluble monomer into various aggregated states [10]. Therefore, preventing the aggregation of Aβ monomer into toxic aggregates may contribute to the prevention and treatment of AD.

Recently, many studies have focused on the discovery and development of compounds capable of inhibiting Aβ fibrillogenesis. Many kinds of inhibitors have been developed to prevent Aβ aggregation, such as small molecules [11–13], peptides [14,15] and nanomaterials [16–18]. Among them, nanoparticles (NPs) have received special interests because they possess high specific surface area, unique structural superiority, ease of surface functionalization and modification [19,20]. Several NPs including polymeric NPs [21], gold NPs [22,23] and magnetic NPs [24], have been used to prevent Aβ fibrillogenesis.

Previous studies have suggested that the structural and surface properties of NPs, especially the surface charge and hydrophobicity, played important roles in the interactions between Aβ and NPs. Due to the intrinsic strong hydrophobicity of Aβ, the exposed hydrophobic areas on NPs would bind Aβ and inhibit its corresponding unfolding and the aggregation process. However, there exists controversy about electrostatic properties of NPs on the inhibitory effect on $A\beta_{42}$. Some studies suggested that positively charged NPs inhibited Aβ fibrillogenesis [25,26]. It is known that Aβ carried net negative charges under physiological condition [27]. The inhibition effect of positively charged NPs on Aβ fibrillogenesis was explained by the tight interaction between NPs and Aβ monomers or growing oligomers, which might decrease free Aβ species, and thus interfere the process of Aβ fibrillogenesis [28]. However, several studies indicated that NPs with

* Corresponding author.
E-mail address: ysun@tju.edu.cn (Y. Sun).

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negative charges had more effective suppressing effects on Aβ aggregation [29,30], although electrostatic repulsion existed between negatively charged NPs and Aβ. For instance, Liao et al. [22] found that negatively charged gold NPs inhibited Aβ fibrillation and greatly reduced the cytotoxicity of $A\beta_{40}$ aggregates. Similar results were reported by Rocha et al. [31]. In addition, Assarsson et al. [32] suggested that positively charged polymers accelerated $A\beta_{42}$ aggregation process, while negatively charged or neutral polymers had no effect on $A\beta_{42}$ aggregation. Moreover, it is noted that all of the above-mentioned studies did not consider the hydrophobicity of the particle surfaces. Thus, the inhibitory effect of surface charges on Aβ fibrillation was complex and the contributions of electrostatic and hydrophobic interactions between NPs and Aβ were poorly understood.

Herein, we have studied the effect of polymeric NPs on Aβ fibrillation by focusing on the effect of surface negative charge density. Firstly, a series of poly *N*-isopropylacrylamide (NIPAm)/acrylic acid (AAc)/N-*t*-butylacrylamide (TBAm) NPs with similar size and equivalent content of hydrophobic groups but different surface negative charge densities were synthesized. Then, their inhibitory effects on $A\beta_{42}$ fibrillogenesis were studied by extensive biophysical and biological analyses. Finally, a mechanistic model for the effect of the NPs on $A\beta_{42}$ fibrillogenesis was proposed to clarify how the negatively charged NPs with surface hydrophobic patches could fight against the fibrillation of amyloid proteins.

2. Material and methods

2.1. Materials

$A\beta_{42}$ (>95%, lyophilized powder) was purchased from GL Biochem (Shanghai, China). 1,1,1,3,3,3-Hexafluoro-2-propanol (HFIP), thioflavin T (ThT), NIPAm, TBAm, AAc, *N*,*N*′-methylene-bis-acrylamide (Bis), 3-(4.5-dimethylthiazol-2-yl)-2.5-diphenyltetrazolium bromide (MTT) and dimethyl sulfoxide (DMSO) were purchased from Sigma (St. Louis, MO, USA). Human neuroblastoma SH-SY5Y cells were obtained from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China). Dulbecco's modified Eagle's medium/Ham's nutrient mixture F-12 (DMEM/F12) and fetal bovine serum (FBS) were obtained from Invitrogen (Carlsbad, CA, USA). All other chemicals were the highest purity available from local sources.

2.2. Preparation of polymeric NPs

Polymeric NPs were synthesized by free radical polymerization in the presence of sodium dodecyl sulfate (SDS). The synthesis procedure followed that reported in literature with minor modifications [18,33]. To prepare NPs with equivalent content of hydrophobic groups but different surface negative charge densities, the polymerization systems contained 2% crosslinker (BIS), 40% TBAm (hydrophobic monomer) and different concentrations of AAc (charged monomer) and NIPAm as listed in Table 1. The solutions were degassed by bubbling with nitrogen for 30 min. Polymerization was induced by adding 60 mg of ammonium persulfate in 1 mL MilliQ water at 25 °C, and the reaction was continued for 15 h under a nitrogen atmosphere. After polymerization, the reaction system was extensively dialyzed against MilliQ water for over 14 days, with the dialysis water changed at least twice per day, until no reactants or SDS could be detected in the dialysis water or NPs solution analyzed by proton NMR (Varian Inova 500-MHz, UT, USA). All spectra were acquired in D_2O. The NPs were freeze-dried and stored at 4 °C before use.

Table 1
Particle size, PDI and zeta potential of the nanoparticles synthesized with the same content of hydrophobic monomer TBAm and different ratios of negatively charged monomer AAc and NIPAm.

NPs	Monomer composition ratio [mol%]		Size in PBS [nm]	PDI	Zeta potential [mV]	
	AAc	NIPAm			In PBS	In water
NP0	0	58	92.0	0.175	−8.9 ± 4.2	−11.8 ± 6.4
NP5	5	53	95.3	0.135	−18.2 ± 5.0	−20.4 ± 6.2
NP10	10	48	94.5	0.134	−24.7 ± 3.7	−33.1 ± 7.2
NP20	20	38	94.0	0.039	−26.8 ± 2.8	−44.1 ± 7.4

2.3. Preparation of $A\beta_{42}$ monomer solution

$A\beta_{42}$ was treated as described in literature [34]. Immediately prior to use, the treated $A\beta_{42}$ was re-dissolved in 20 mM NaOH, and sonicated for 20 min. After centrifugation (16,000*g*) for 30 min at 4 °C, the upper 75% of the supernatant was carefully collected and diluted with phosphate buffered saline (PBS) solution (10 mM sodium phosphate containing 100 mM NaCl, pH 7.4) to the final protein concentration of 25 μM.

2.4. Thioflavin T fluorescent assay

$A\beta_{42}$ fibrillization was monitored by the ThT dye-binding assay. ThT fluorescence was performed using a fluorescence spectrophotometer (Perking Elmer LS-55, MA, USA) at 25 °C with excitation at 440 nm, emission at 480 nm and slit width of 5 nm. The results were reported by the average of three measurements. The standard deviations were calculated and represented as error bars in the corresponding figures. For the assays the solutions were incubated at 37 °C by continuous shaking at 160 rpm. Aliquots of the incubation solutions at different time intervals were then diluted 11 times into ThT solution (25 mM ThT in PBS) for measurements.

2.5. Dynamic light scattering

The size distributions of $A\beta_{42}$ aggregates and NPs were analyzed using Zetasizer Nano (Malvern Instruments, Worchestershire, UK). The samples were prepared by incubating 25 μM $A\beta_{42}$ with different amounts of NPs in a shaking incubator at 160 rpm and 37 °C. The temperature effect on the particle size of NPs was also analyzed by dynamic light scattering (DLS). The temperature of the NP samples was controlled with a Peltier device at a range from 4 °C to 37 °C. All the DLS results were reported as the average of three measurements.

2.6. Transmission electron microscopy

The morphologies of $A\beta_{42}$ fibrils and NPs were observed by transmission electron microscopy (TEM). Aβ samples were prepared using the method described previously [35] and the stained $A\beta_{42}$ samples were examined and photographed using a JEM-100CXII transmission electron microscope system (JEOL Inc., Tokyo, Japan) with an accelerating voltage of 100 kV. The length distributions of amyloid fibrils were also obtained by measuring the fibril lengths from TEM images.

2.7. Cell viability assay

The MTT assay was employed to determine the cell viability using human neuroblastoma SH-SY5Y cell line [36,37]. SH-SY5Y cells were cultured in DMEM medium with 20% FBS, 2 mM L-glutamine, 100 U/mL penicillin and 100 μg/mL streptomycin. Cells were seeded at a density of 5×10^3 cells per well in a 96-well plate. After 24 h incubation in 5% CO_2 atmosphere in 37 °C incubator (MCO-18AIC, Sanyo Electric, Japan), the solutions with different concentrations of NPs were added into the plate, followed by 25 μM freshly prepared $A\beta_{42}$ stock solutions (in PBS) in the inhibition experiments. After further incubation for 24 h, MTT was added to each well at a final concentration of 0.5 mg/mL and incubated for another 3 h. The medium was discarded and 100 μL DMSO was added to each well. Finally, the absorbance at