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Two-in-one polydopamine nanospheres for fluorescent determination of beta-amyloid oligomers and inhibition of beta-amyloid aggregation

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ABSTRACT

Beta-amyloid (Aβ) oligomers are the reliable molecular biomarkers and crucial targets for the diagnosis and therapy of Alzheimer's disease (AD). In this work, we reported on a fluorescent platform for the selective detection of Aβ oligomers with polydopamine nanospheres (PDANS) as the nanoquencher. The carboxyfluorescein (FAM)-labeled DNA aptamers specific to Aβ oligomers showed poor fluorescence signal when absorbed onto the surface of PDANS. The specific binding of Aβ oligomer to the FAM-DNA caused the conformation change of the aptamer into a hairpin structure, thus inducing the release of FAM-DNA from the PDANS surface and making the fluorophore far away from the nanoquencher. Aβ oligomers were thus determined based on the increase in the fluorescence signal. The "signal on" fluorescent platform could be used for sensing of Aβ oligomers, but not for the monomers and fibrils. The detection limit was found to be 12.5 nM. More intriguingly, we found that PDANS efficiently prevented the aggregation of Aβ monomers. We believe that the two-in-one PDANS as both the nanoquencher and the inhibitor would found many applications for determination of Aβ oligomers in bodily fluids and drug-therapy of AD.

1. Introduction

Alzheimer's disease (AD), the most common neurodegenerative disorder, will afflict more than 66 million of the population globally by the year 2030. Clinically, the neurologic disorder is characterized by problem with language, disorientation, mood swing, loss of motivation, not managing self care, and behavioral issues. Substantial investigations have clearly demonstrated that the abnormal aggregates of beta-amyloid (Aβ) peptides in brains play a critical role in the etiology of AD [1]. A monomeric Aβ peptide is comprised of 39–43 amino acid residues, resulting from proteolytic cleavage of amyloid precursor protein (APP) by β- and γ-secretase. However, Aβ monomers can multimerize into soluble Aβ oligomers, and further progress directly into higher molecular weight Aβ fibrils. There are good evidences that soluble Aβ oligomers, comprising of 50–100 Aβ monomers, are highly toxic to neuronal cells in preclinical AD [2–4]. Also, the elevated levels of Aβ oligomers have been found in the cerebrospinal fluids of AD patients [5,6]. Thus, Aβ oligomers have been considered as the therapeutic targets and the diagnostic markers. Initially, organic dyes, such as cyanine dyes and anilinonaphthalene sulfonate dyes have been used to monitoring the process of Aβ assembly and determining the oligomeric and fibrillar Aβ species [7–11]. However, most of the dyes cannot discriminate Aβ oligomers from the other β-sheets aggregates including Aβ fibrils. This is detrimental to the accuracy for the quantitative assay of Aβ oligomers. Therefore, it has been desired to develop a selective fluorescent platform for the sensitive determination of Aβ oligomers.

Homogeneous "signal on" fluorescence assays have attracted tremendous attention owing to their high sensitivity, rapid response time and operation convenience. The development of fluorescent labeling of facile selection of aptamers has opened the door that enables nucleic acid probes to be widely used in a broad spectrum of applications [12–14]. Molecular beacon (MB), which is labeled with a fluorescent donor and a fluorescent accepter, has been widely applied to determine various molecules [15]. Compared with traditional MBs, the introduction of nanoquenchers can eliminate the difficult selection of a fluorophore-quencher pair and improve the signal-to-noise ratio of the assay through fluorescence resonant energy transfer or electron transfer [16]. Over the past few years, various nanomaterials have been used as the nanoquenchers for designing of fluorescent chem-/bio-sensors because of their excellent optical and physical property, small size, and high surface-to-volume ratio, typically including gold nanoparticles (AuNPs), graphene oxide (GO), carbon nanospheres (CNSs), carbon nanotubes (CNTs), two-dimensional transition metal sulfide

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nanosheets (e.g. MoS_2 and WS_2), and metal-organic frameworks [17–21]. Unfortunately, as for most of the nanoquenchers, cytotoxicity remains a concern for biomedical applications [21]. As generally known, dopamine can be oxidized and self-polymerized to form polydopamine under a weak alkaline condition. Polydopamine has been widely applied in coating materials, drug release, photothermal therapy and immunoassays [22,23]. In particular, polydopamine nanospheres (PDANS) possess obvious merits such as uniform size distribution, facile synthesis, excellent compatibility with biomolecules and low toxicity. For example, they can tightly absorb single stranded DNA (ssDNA) by hydrogen bonding and π–π stacking interactions between the nucleobases of ssDNA and the aromatic groups of polydopamine. Because of the distinct broad-band absorbance in the UV–vis spectrum, polydopamine nanostrutures present wide potential applications as the fluorescence nanoquenchers to develop novel biosensing platforms for the detection of biomolecules (e.g. ATP, miRNA, protein and DNA) and cells [19–21,24–29]. Typically, Dong's group investigated the fluorescence quenching efficiency of polydopamine nanotubes (PDANTs) toward various fluorescent dyes and demonstrated their applications in fluorescent biosensing [19]. Sun's group and Xu's group demonstrated that the nonocomplexes of PDANS/DNA can be used as the sensing probes for the detection of ATP, DNA and thrombin [21,27]. Zhang and co-workers evaluated antioxidants in complex biological fluids by utilizing the antioxidants to block the polymerization reaction of dopamine, thus preventing the PDANS-triggered quenching of fluorescently labeled ssDNA [29]. However, to the best of our knowledge, no effect has been made on the PDANS-based diagnosis and therapy of AD.

Aptamer is an excellent example of functional bioreceptors selected in vitro. Ikebukuro and co-workers have isolated the Aβ oligomer-specific DNA aptamers (K_d = 25 nM) by the combination of gel-shift assay and competitive screening method [30]. The selected aptamers have been successfully used for evaluating the level of Aβ oligomers with a molecular beacon (MB) fluorescence system and an antibody-aptamer sandwich electrochemical immunoassay [31,32]. In the present work, we found that the carboxyfluorescein (FAM)-labeled DNA aptamers can be used for the selective detection of Aβ oligomers with PDANS as the nanoquencher. More intriguingly, we found that PDANS prevented the aggregation of Aβ. This work thus presents a very promising tool for the diagnosis and therapy of AD.

2. Experimental

2.1. Chemicals and reagents

Carboxyfluorescein (FAM)-labeled single-stranded DNA (ssDNA) probe with a sequence of GGTGGCTGGAGGGGGCGCGAACG (FAM-DNA) and dopamine hydrochloride were obtained from Sangon Biotech. Co., Ltd. (Shanghai, China). The Aβ monomer with a sequence of DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVV, bovine serum albumin (BSA), lysozyme, myoglobin, IgG, thioflavine T (ThT), and 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP) were purchased from Sigma–Aldrich (Shanghai, China). All other chemicals were of analytical grade and obtained from Beijing Chemical Reagent Co. Ltd. (Beijing, China). The water used in all experiments was prepared by a Millipore system (Simplicity Plus, Millipore Corp.).

2.2. Instruments

Scanning electron microscopy (SEM) images were recorded using a SU8010 scanning electron microscope (Hitachi, Japan). Fluorescent emission spectra were recorded on a Varian Cary fluorescence spectrometer. Atomic force microscopy (AFM) images were obtained on a Dimension Edge microscope (Bruker Nano Inc., Santa Barbara, CA) equipped with a tapping mode.

2.3. Preparation of PDANS

The PDANS with a average diameter of ~80 nm were synthesized according to the previously reported procedure with slight modifications [33]. 180 mg of dopamine hydrochloride was dissolved in 90 mL of water. Under vigorous stirring, 760 μL of 1 N NaOH solution was added to the dopamine solution at 50 °C. The color of the solution gradually turned to pale yellow with the addition of NaOH and finally changed to dark brown. After keeping stirring for 5 h, the suspension was centrifuged by 14 000 rpm and washed/resuspended with deionized water three times. After the large-size impurity was removed by 4000 rpm, the powder of PDANS was received with vacuum freeze drying equipment and stored at −4 °C.

2.4. Preparation of Aβ oligomers

The soluble Aβ oligomers were prepared and characterized with the procedures as reported in our previous works [34,35]. Briefly, Aβ sample was first dissolved in HFIP at a concentration of 1.0 mg/mL^{-1} and then sonicated at 25 °C for 10 min. After removing HFIP by a gentle stream of argon gas, the sample was dissolved in 20 mM NaOH and then diluted to 100 μM with phosphate buffer solution (20 mM, pH 7.4). With the increase of incubation time, the Aβ monomers assembled into soluble Aβ oligomers and fibrils. The concentration of Aβ oligomer/fibril was expressed as the equivalent one to the monomer.

2.5. Fluorescence assays

To determine the fluorescence quenching efficiency of PDANS, 100 μL of FAM-DNA was mixed with 100 μL of PDANS at a given concentration, followed by the addition of 800 μL PBS to incubate for 10 min at room temperature. For the assay of Aβ oligomers, 100 μL Aβ sample at a desired concentration was added into 100 μL of the mixed PDANS/FAM-DNA suspension and incubated at room temperature for 40 min.

2.6. Inhibition of Aβ aggregation

To evaluate the inhibition ability of PDANS to Aβ aggregation, the freshly prepared Aβ sample was incubated with PDANS suspension at different concentrations. The aggregation of Aβ in the absence and presence of PDANS was monitored by a standard ThT-binding assay. The fluorescence signal (excitation at 450 nm) was recorded between 460 and 600 nm by using 10 nm slits. After incubation for 96 h, the samples were taken out for the SEM characterization.

3. Results and discussion

3.1. Principle of the sensing platform

The principle of the sensing platform for the selective assay of Aβ oligomers was shown in Scheme 1. The FAM-DNA aptamers can adsorb onto the surface of PDANS through the π–π stacking interactions. The resulting PDANS/FAM-DNA nanocomplexes showed low fluorescence signal due to the prominent nanoscale-surface energy transfer effect from FAM to PDANS. With the addition of Aβ oligomers, the specific binding between Aβ oligomer and FAM-DNA will cause the conformation change of the aptamer into a hairpin structure [30], thus weakening the interaction between FAM-DNA