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Aptasensors for the selective detection of alpha-synuclein oligomer by colorimetry, surface plasmon resonance and electrochemical impedance spectroscopy

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ABSTRACT

Soluble alpha-synuclein (α-syn) oligomer is believed to be a reliable molecular biomarker for diagnosis of Parkinson's disease (PD). Thus, it is critical to develop a simple method for the selective detection of α-syn oligomer with low cost as well as high sensitivity. In this paper, we reported the label-free detection of α-syn oligomer using a DNA aptamer as the bioreceptor with the techniques of gold nanoparticles (AuNPs)-based colorimetric assay, surface plasmon resonance (SPR) and electrochemical impedance spectroscopy (EIS). In the colorimetric assay, the aptamer adsorbed onto the surface of AuNPs to prevent the salt-induced aggregation of the nanoparticles. However, the specific binding of α-syn oligomer to aptamer prevented the absorption of aptamer onto the surface of AuNPs. As a result, the aggregation of AuNPs was triggered by high concentration of salt with a color change from red to blue. In the SPR- or EIS-based surface analysis, specific binding of α-syn oligomer to the aptamer immobilized onto the gold film or electrode surface led to an increase in the SPR dip shift or the electron-transfer resistance. The detection limits of the colorimetry, SPR and EIS were found to be 10 nM, 8 pM and 1 pM, respectively. The amenability of this method to α-syn oligomer analysis in a biological matrix was demonstrated by assay of α-syn oligomer in serum.

1. Introduction

Parkinson's disease (PD), the second most common neurodegenerative disorder after Alzheimer's disease, affects approximately seven million people globally. A hallmark of PD is that surviving dopaminergic cells contain cytosolic filamentous inclusions known as the Lewy bodies [1,2]. A major component in Lewy bodies is the alpha-synuclein (α-syn) aggregates, whose monomeric constituent is a synuclein protein of unknown function primarily found in neural tissue [1,3,4]. α-Syn monomer makes up as much as 1% of all proteins in the cytosol of brain cells. However, it can aggregate first into small oligomeric species and then into higher molecular weight fibril [5,6]. Among them, soluble oligomer is viewed as primarily neurotoxic and responsible for neuronal death in preclinical PD [7–11]. Also, the elevated levels of α-syn oligomer has been detected in the cerebrospinal fluid and plasma of PD patients [12–19]. Thus, α-syn oligomer has been considered not only as the therapeutic target but also as the diagnostic marker [20–24]. Recently, a few new methods have been developed for the detection of α-syn monomer with improving sensitivity, such as electrochemical immunosensors [25,26], mass spectrometry [27], fluorescent immunoassay [28], and capillary electrophoresis [29,30]. These methods are feasible in laboratory studies, but expensive, labor-intensive and/or less specific for clinical assays. Moreover, assay of α-syn monomer only might be unable to discriminate between PD patients and healthy controls or other types of dementia because the levels of α-syn monomer may differ by gender and age [31,32]. In view of the high neurotoxicity of α-syn oligomer in PD, the direct detection of α-syn oligomer would be more reliable for PD diagnosis than assay of α-syn monomer [29,33].

Currently used method for clinical detection of α-syn oligomer is the enzyme-linked immunosorbent assay (ELISA) [12–19]. However, this method requires the relatively expensive and less stable antibody for molecular recognition. Moreover, the reported antibody of α-syn oligomer would also recognize α-syn monomer and other α-syn aggregates and metabolites to some extent

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[21,34]. Additionally, the organic dyes-based fluorescence assays (e.g. thioflavin T or ThT) have been commonly used for monitoring the formation of α-syn aggregates in laboratory investigations. However, most of the dyes cannot be used to discriminate α-syn oligomer from other β-sheets of α-syn aggregates, which is detrimental to the accurately quantitative assay of α-syn oligomer [8,35–38].

Aptamer is an excellent example of functional bioreceptors selected *in vitro*. After nearly 30 years' endeavor, DNA and RNA aptamers have been identified as binding tightly to a broad range of targets (e.g., proteins, peptides, amino acids, drugs, metal ions and even whole cells) [39–42]. Because of its advantageous characteristics over antibody (e.g., small sizes, long-term stability, simple preparation procedure and low preparation cost), aptamer has emerged as a good alternative to antibody in the design of novel electrochemical, optical and mass-sensitive biosensor devices that have exhibited high sensitivity and selectivity [43,44]. Recently, Ikebukuro's group has selected a DNA aptamer that strongly bound to α-syn oligomer but not to α-syn monomer or fibril [34]. The dissociation constant (K_d) of the aptamer for α-syn oligomer was estimated to be 60–70 nM [34]. This value is close to that between antibody and α-syn monomer [26], suggesting that the aptamer shows a high binding affinity to α-syn oligomer. In the present work, we attempted to develop a label-free aptasensor for the selective detection of α-syn oligomer. Among kinds of aptasensors, metal nanoparticles-based liquid-phase colorimetric assay has received considerable attention as it enables color visualization without a specific instrument [45]. Surface plasmon resonance (SPR) has also been shown as a promising technique for analyte concentration determination and kinetic studies of biomolecular interactions due to its attractive features, such as high sensitivity, label-free and real-time measurements, and relatively simple procedure [46–50]. Furthermore, for assay of non-electroactive protein in a single label-free and quantitative manner, electrochemical impedance spectroscopy (EIS) is particularly powerful because of its inherently favorable attributes of high innate sensitivity, facile miniaturization, low detection cost, and less sample consumption [51,52]. For this consideration, herein, we reported the label-free detection of α-syn oligomer using a DNA aptamer as the bioreceptor by gold nanoparticles (AuNPs)-based colorimetric assay, SPR and EIS. The analytical performances of the three aptasensors were compared and their practical applications for assays of α-syn oligomer in serum were demonstrated.

2. Experimental

2.1. Chemicals and reagents

α-Syn monomer, bovine serum albumin (BSA), immunoglobin G (IgG), lysozyme, thrombin, 6-mercapto-1-hexanol (MCH), tris(carboxy-ethyl)phosphine (TCEP), serum, trisodium citrate, KH_2PO_4 and K_2HPO_4 were purchased from Sigma-Aldrich. The α-syn oligomer-specific aptamers (5′-TTTTTGGTGGCTGGAGGGGGCGCGAACG-3′ and 5′- HS-$(CH_2)_6$-TTTTTGGTGGCTGGAGGGGGCGCGAACG-3′) were synthesized and purified by Sangon Biotech. Co., Ltd. (Shanghai, China). All other chemicals were of analytical grade and obtained from Beijing Chemical Reagent Co., Ltd. (Beijing, China). All solutions were prepared with ultrapure water from a Millipore system.

The citrate-stabilized AuNPs with a size of 13 nm were prepared using a trisodium citrate reduction method and diluted with 2 mM phosphate-buffered saline solution (PBS buffer, pH 7.2). The particle concentration of the AuNPs suspension was determined based on a molar absorptivity of 2.7×10^8 M^{-1} cm^{-1} at 520 nm. The preparation of α-syn oligomer follows the reported procedure [5]. In brief, α-syn monomer was dissolved in the 2 mM PBS, filtered with 0.2 μm filter membrane and then incubated at room temperature for a given time. The concentration of α-syn monomer was determined by absorption measurement with a NanoDrop spectrophotometer (ND-1000, Thermo Scientific) using a theoretical extinction coefficient of 5960 M^{-1} cm^{-1}.

2.2. Colorimetric assay

50 μL of aptamer solution was first mixed with 50 μL of α-syn oligomer at a given concentration to react for 10 min. Then, 170 μL of AuNPs was added to the mixture. After 5-min incubation, 30 μL of PBS containing 0.8 M NaCl was added to the suspension. After reaction for 10 min again, the color change of the mixed solution was recorded by a digital camera. The absorbance spectra were collected with a Cary-60 UV–vis spectrophotometer.

2.3. SPR detection

The immobilization of aptamer on the SPR sensing chips was carried out based on the Au-S interaction. Briefly, the cleaned Au films provided by Biosensing Instrument Inc. (Tempe, AZ) were immersed in the mixed solution of 10 μM thiolated aptamer (5′- HS-$(CH_2)_6$-TTTTTGGTGGCTGGAGGGGGCGCGAACG-3′), 50 μM TCEP and a given concentration of MCH overnight to form the aptamer/MCH self-assembled monolayers (SAMs). Upon the completion of the surface modifications, the resultant chips were rinsed with ethanol and deionized water, dried with nitrogen and then stored at 4 °C for use. For the α-syn oligomer detection, the prepared sensing chip was assembled onto the SPR instrument (BI-SPR 3000, Biosensing Instrument Inc., Tempe, AZ) for measurements. After a stable baseline was obtained, the α-syn sample was delivered onto the SPR flow cell using a syringe pump.

2.4. Impedance analysis

The cleaned gold electrode with a diameter of 2 mm was incubated with a PBS solution containing 10 μM thiolated aptamer, 50 μM TCEP and 1.2 mM MCH overnight. After thoroughly washing the electrode with ethanol/water, 10 μL of α-syn sample at a given concentration was cast onto the sensor surface for 30 min. After having been rinsed with water again, the electrode was placed in a mixed solution of 5 mM $[Fe(CN)_6]^{3-/4-}$ (1:1) and 0.1 M KCl for impedance measurement on a CHI 660E (CH Instruments, Shanghai, China) electrochemical workstation. Electrochemical impedance spectroscopy was collected at the potential of 0.245 V in the frequency range of 0.01–500 kHz. The auxiliary electrode and the reference electrode were platinum wire and Ag/AgCl, respectively. For the signal-amplified detection, the aptamer-covered electrode was incubated with the α-syn sample, washed with water, and then exposed to 50 μL of AuNPs suspension. After having been washed thoroughly with water, the electrode was placed in the solution of $[Fe(CN)_6]^{3-/4-}$ for impedance measurement.

3. Results and discussion

3.1. Colorimetric assay

Since the report of the most classical colorimetric sensor proposed by Mirkin's group [53], AuNPs have shown tremendous potential in colorimetric aptasensors because of their high extinction coefficients and distance-dependent optical properties. Usually, AuNPs-based aptasensors can be divided into two categories: DNA-functionalized AuNPs aptasensors and unfunctionalized AuNPs aptasensors. In the latter, aptamer could bind to the unmodified AuNPs and stabilize them against salt-induced