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Label-free detection of ApoE4-mediated β-amyloid aggregation on single nanoparticle uncovering Alzheimer's disease

Min Kyung Kang [a], Jeewon Lee [a], Anh H. Nguyen [a], Sang Jun Sim [a,b,*]

[a] Department of Chemical and Biological Engineering, Korea University, Seoul 136-713, Republic of Korea
[b] Green School, Korea University, Seoul 136-713, Republic of Korea

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ABSTRACT

Beta amyloid (Aβ) deposition is a pathological milestone of Alzheimer's disease (AD). This is facilitated by an isoform of Apolipoprotein E4 (ApoE4), which is a dominant risk factor for AD. However, current *in vitro* Aβ aggregation assays were performed in extreme conditions not linked to physiological conditions, to understand the mechanism of Aβ induced neurotoxicity. Here, we present a simple method for the ApoE4-mediated Aβ aggregation at physiological conditions using single gold nanoparticle based on localized surface plasmon resonance (LSPR). It can be directly observed by dark-field microscope or even by the naked eye. Following LSPR principles, we used ApoE4 inducing Aβ42 self-assemblies on gold nanoparticles (AuNPs) surface *via* their surface charge interaction. Using physiologically mimic cerebrospinal fluid, we determined a detection limit of 1.5 pM for Aβ42 corresponding to the ~2.9 nm LSPR-peak shift under ApoE4. Interestingly, the result also shows that ApoE4 induces the aggregation of Aβ42 more specifically and rapidly than that of Aβ40. This is the first biomimetic platform for real-time detection of Aβ aggregation, mimicking biological conditions, which can be used to investigate AD directly.

1. Introduction

Aggregation of amyloid β (Aβ) is a well-known pathological milestone of AD which leads to the formation of neutric plaques in the brain. Recent data prove that the ApoE4 involves both the stimulation of Aβ deposition and the inhibition of Aβ clearance, leading to plaque formation (Wisniewski and Blas, 1992). Consequently, development of competent methods to monitor the structured growth of Aβ from monomer to fibrillar aggregation at physiological conditions based on the interaction of ApoE4 and Aβ could provide a real disease mechanism and AD diagnostics. Currently, methods of Aβ detection are based on extreme conditions such as high ionic conditions and fluorescence labeling, or label-modified methods (Thiolflavin T and Congo red) (Campbell et al., 1987). Although these methods are quite sensitive, they do not reflect Aβ deposition in physiological conditions in which ApoE4 plays as a crucial role.

LSPR is generated by the collective electrons of an AuNP oscillating with incident photons (Kedem et al., 2011). LSPR is sensitive to alterations in the local dielectric environment, which is dependent on the local electric field from the binding of various analysts, such as protein or peptide assemblies (Ma et al., 2015). Previously, we have detected the protein–DNA interaction with nanoplasmonic sensors (Song et al., 2013). Using dark-field microscope, the individual gold nanoparticle can be used to measure the binding of Aβ assembly on the nanoparticle's surface using LSPR.

The single AuNP as a sensing element can act as a nucleation site for Aβ assembly under ApoE4 activity because the nanoparticle surface offers a preferable interface for self-assembly catalysis (Reches and Gazit, 2006). As a result, single AuNP can become a sensing element and catalytic site to forward Aβ self-assembly and to allow quick tracking of the aggregation; this is expected more efficient than the case of bulk colloids in high ionic conditions.

Plasmonic nanoparticles are advancing sensing technology. Exploitation of light scattering from gold nanoparticles (AuNPs) can be effectively optically reported with biosensor to monitor dynamic interaction at physiological conditions (with no photo bleaching, but enhanced scattering and biocompatibility) (Sönnichsen et al., 2005). Localized surface plasmon resonance (LSPR) offers many new opportunities for detection of self-assembly of proteins and Aβ deposition on single AuNPs by measuring refractive index changes surrounding single AuNPs. Recent studies have shown that AuNPs can monitor Aβ aggregation under chemical perturbation conditions (Choi and Lee, 2013). These studies recognize the presence of Aβ in the samples and indirectly implicate amyloid conformational disease. However, the presence of Aβ sometime does not link to AD disease since it also depends

* Corresponding author at: Department of Chemical and Biological Engineering, Korea University, Seoul 136-713, Republic of Korea. Fax: +82 2 926 6102.
E-mail address: simsj@korea.ac.kr (S.J. Sim).

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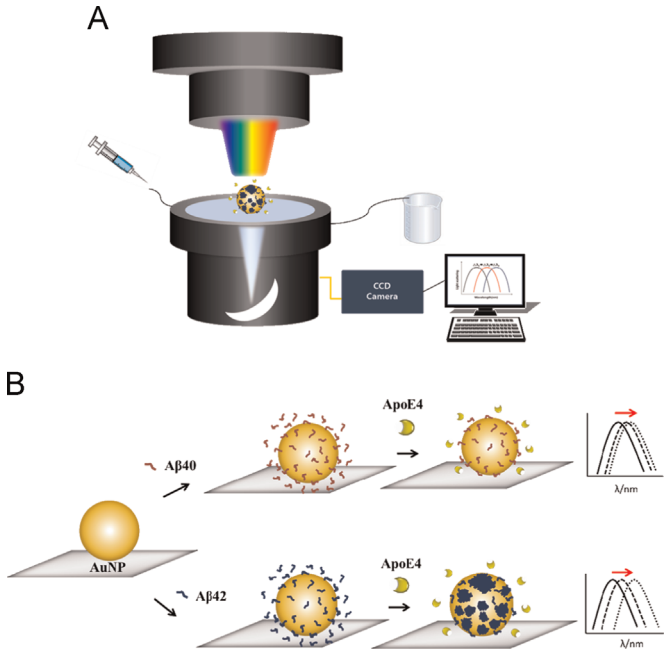

Scheme 1. Fabrication of the sensor. (A) Schematic of the overall process describing the system setup by LSPR. (B) Experimental process for binding affinity.

on ApoE4 at physiological conditions.

Aβ aggregation at physiological conditions with ApoE4 is a crucial process highly associated with Aβ aggregation in AD disease. Here we used Rayleigh scattering to study Aβ42 aggregation based on ApoE4-mediated aggregation on a single gold nanoparticle (Scheme 1A). The mechanisms of Aβ nucleation, aggregation, and deposition, which are followed by reversible deposition of both Aβ42 and Aβ40, along with growth of the deposits and their upcoming irreversible fibrillation, were studied. Interestingly, this design allowed detecting real-time Aβ plaque formation, fibrillation growth, and ApoE4-mediated Aβ deposition on a single nanoparticle surface using LSPR, which may reflect early pathological interactions of Aβ with ApoE4 isomers.

2. Materials and methods

2.1. Materials

Hydrogen tetrachloroaurate (III) trihydrate ($HAuCl_4 \cdot 3H_2O$), sodium citrate, absolute ethanol, 3-aminopropyltriethoxysilane (APTES), bovine serum albumin (BSA), β-amyloid (1–40), and β-amyloid (1-42), Sodium chloride (NaCl), Potassium chloride (KCl), Calcium chloride dihydrate ($CaCl_2 \cdot 2H_2O$), Magnesium chloride hexahydrate ($MgCl_2 \cdot 6H_2O$), Sodium monohydrogen phosphate heptahydrate ($Na_2HPO_4 \cdot 7H_2O$), Sodium dihydrogen phosphate ($NaH_2PO_4 \cdot H_2O$) were purchased from Sigma Aldrich (Korea), Apolipoprotein E4 (ApoE4) was obtained from Biovision. Ultrapure water (18.2 $m\Omega\,cm^{-1}$) was used to prepare all of the chemical solutions.

2.2. Synthesis of gold nanoparticles

Gold nanoparticles (AuNPs) were synthesized through a sodium citrate reduction of an aqueous $HAuCl_4$ solution. Briefly, 10 mL of 1.0 mM $HAuCl_4$ was brought to a boil, 1.0 mL of 0.4% sodium citrate was then added with vigorous stirring. The solution was boiled for 5 min to complete the citrate reduction of the gold ions. The solution was then stirred (700 rpm) for 15 min, and then it cooled to room temperature for 15 min. The solution was subsequently filtered with a 0.2 μm filter to remove aggregated particles. The UV–vis absorption spectra of the gold solution were recorded using UV–vis spectrophotometer (Shimadzu UV3600 UV–vis-NIR). The size and morphology of the synthesized AuNPs were estimated by transmission electron microscopy (HRTEM, JEOL JEM-3011 operated at 300 kV).

2.3. Fabrication of the sensor

The experimental process using the individual AuNP sensors is described below (Scheme 1B). A glass slide ($22 \times 40 \times 0.1\ mm^3$) was first cleaned in acetone by sonication for 30 min and rinsed thoroughly with absolute ethanol 3 times. Then, the glass was cleaned in a piranha solution (3:1 H_2SO_4: H_2O_2), incubate for 30 min, and rinsed thoroughly with distilled water and absolute ethanol. The cleaned slide glass was subsequently dried with nitrogen gas, and the surface was treated in 2% (v/v) APTES in 99.9% absolute ethanol for 20 min. For sensor fabrication, 10 μL 50-nm AuNPs solution (maximum absorption wavelength: 531 nm, OD: 0.04) was drop-coated onto the modified glass. The prepared glass slide was mounted on a closed confocal imaging chamber (RC-30, Warner Instrument Inc.), and inserted into the sample holder of a dark-field microscope (Eclipse TE2000-U, Nikon, Japan) to observe dispersed AuNPs before sensing. Fluidic flow inside the chamber was fixed at 100 $\mu L\ min^{-1}$ by a syringe pump. The Rayleigh light scattering spectra of individual gold nanoparticles were measured by dark-field microscopy (Eclipse TE2000-U, Nikon, Japan), a spectrograph (Microspec 2300i, Roper Scientifics), and Charge Coupled Device (CCD) camera (PIXIS: 400B, Princeton Instruments). The system was used to observe the scattered light of the single gold nanoparticles, a transmission configuration of the monochromatic light, which was recorded as a function of light scattering (intensity versus wavelength), for the resonant Rayleigh light scattering micro-spectroscopy; further details of this system have been previously reported (Nguyen and Sim, 2015). The distinct Rayleigh scattering from specific events of beta amyloid aggregation on the sensor was recorded. The plasmon shift ($\Delta\lambda_{max}$) of the LPSR spectra of the events occurring on the sensor was then analyzed with the following formula: $\Delta\lambda_{max} = \lambda_{max}$ (after aggregation) $- \lambda_{max}$ (before aggregation) after fitting with the Lorentzian algorithm.

2.4. ApoE4-mediated β amyloid aggregation

To measure ApoE4-mediated Aβ42 aggregation on the individual AuNP surface, a mixture of ApoE4 (10 nM) and Aβ42 (50 μM) was injected into the biosensor in Cerebrospinal fluid (CSF) buffer (pH 7.4); Preparation of solution A: Appropriate amounts of each compound listed below were weighed and dissolved in 500 ml pyrogen-free, sterile water (NaCl 8.66 g, KCl 0.224 g, $CaCl_2 \cdot 2H_2O$ 0.206 g, $MgCl_2 \cdot 6H_2O$ 0.163 g). Preparation of Solution B: Appropriate amounts of each compound listed below then weighed and dissolved in 500 ml pyrogen-free, sterile water ($Na_2HPO_4 \cdot 7H_2O$ 0.214 g, $NaH_2PO_4 \cdot H_2O$ 0.027 g). Solutions A and B were combined in a 1:1 ratio, and incubated overnight. After this reaction, the chamber was washed to eliminate non-aggregated Aβ42 from the sensor and to release ApoE4. Rayleigh scattering spectra of peptide Aβ42 aggregation was recorded and analyzed as described in Section 2.3.