Talanta 139 (2015) 35–39

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Coomassie brilliant blue R-250 as a new surface-enhanced Raman scattering probe for prion protein through a dual-aptamer mechanism

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ARTICLE INFO

Article history:
Received 29 October 2014
Received in revised form
27 December 2014
Accepted 30 December 2014
Available online 8 January 2015

Keywords:
Prion protein
Aptamer
Surface-enhanced Raman scattering
Ag@Si NPs
Protein labeling

ABSTRACT

Surface-enhanced Raman scattering (SERS) spectra, which can provide large information about trace amount of chemical and biological species have been widely performed as a well-established tool in complex biological system. In this work, coomassie brilliant blue (R-250) with high affinity to proteins and high Raman activity was employed as a Raman reporter to probe prion protein (PrP) through a dual-aptamer mechanism, and thus an original strategy for PrP determination was proposed, which showed great potential to turn on the SERS response through specific recognition of anti-prion aptamers towards the target protein. Aptamers (Apt1 and Apt 2) recognizing distinct epitopes of PrP with high affinity were first conjugated to Ag@Si NPs, and Ag@Si-PrP/R-250-Ag@Si conjugates were obtained in the presence of PrP/R-250, inducing dramatically enhanced Raman signal. SERS responses enhanced with increasing amount of PrP and a linear equation of $I_{SERS}=6729.7+3091.2\ c_{PrP}$ was obtained in the range of 3.0–12.0×10^{-9} M with the determination coefficient of 0.988. The proposed strategy is simple, rapid, and high specificity to probe protein–aptamer recognition in the solution.

1. Introduction

Prion diseases or transmissible spongiform encephalopathies (TSEs), are a group of fatal neurodegenerative disorders that affect the nervous system in human and animals [1]. In the past decades, the emergence of variant Creutzfeldt–Jakob disease (vCJD), a kind of prion disease happened in human, has put prions in the spotlight due to its potential epidemic in human [2–3]. The causative event of TSEs is believed to be the conformational conversion from cellular form (PrP^{C}) to disease-causing isoform (PrP^{Sc}) [4], which can accumulate in the brain and lead to disease pathogenesis [5]. Although these rare but unique neurodegenerative disorders have attracted so much attention, a lot of questions remain far from clear, and a simple determination method of prions is meaningful and compulsory. Recently, Surface-enhanced Raman scattering (SERS) spectra, which can provide large information on the chemical structure of the probed substances, have been successfully performed as a well-established tool in complex biological system [6–8]. SERS-based method as a sensitive analytical technique has been used to investigate the prion protein expression and PrP^{C}–Cu(II) interaction [9] and rapid detection of scrambled prions [10] in complex biological media. In addition, with its high sensitivity and selectivity, SERS spectroscopy can be applied for protein–ligand interactions without any label or fixation [11].

Since the discovery of aptamer, it has been widely used as an excellent ligand to recognize prions [12], and many high sensitive detection methods have been achieved [13–14]. Aptamer is a type of DNA or RNA oligonucleotides that can bind to various molecular targets such as small molecules, proteins, nucleic acids even cells, tissues and organisms [15]. Compared to the traditional specific lock-key (antibody–antigen) recognition, aptamer has its own advantages, including easy preparation, good stability, reusability, high affinity and selectivity. As a result, aptamers have been widely applied in molecular recognition [16], cancer diagnosis [17], protein detection [18] and imaging in vivo [19]. PrP has been reported possessing two distinct binding epitopes for two aptamers. One aptamer (Apt1) recognizes epitope 23–90 of the N-terminal [20], while the other one (Apt2) specifically binds with the 90–231 of prion, which is corresponded to the β-sheet structure of PrP [21]. With the involvement of both of the anti-prion aptamers, which possess high affinity to the target, a high selective strategy was proposed to investigate the prions–aptamer interaction.

Coomassie dyes, which have been widely used to stain proteins [22], can bind to proteins with high affinity by physisorption to aromatic amino acids and other amino acids (e.g.: Arginine and

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http://dx.doi.org/10.1016/j.talanta.2014.12.050
0039-9140/

Proline) [23], and it has been of quite importance for protein analysis due to its convenience and high sensitivity. Considering the properties of the dyes and their strong Raman activity, brilliant blue R-250 was employed to label the target proteins and applied as the Raman reporter. Recently, increasing experimental evidence demonstrates that metallic nanoparticles, especially gold or silver nanoparticles, were reported possessing resonant excitation of plasmons [24–26], while assembly of the nanoparticles has been applied as ideal SERS substrates due to their optical excitations known as plasmon resonance scattering (PRS) properties [27–29]. Considering the unique properties of silver nanoparticle and excellent biocompatibility of silica, core–shell nanoparticle architecture-silica coated silver (Ag@Si) NPs were utilized in this work for the immobilization of target proteins as well as the SERS enhancement substrate and an original PrP quantitative employing coomassie brilliant blue (R-250) as Raman reporter and protein labeling.

2. Material and methods

2.1. Materials

Two aptamers of anti-prion protein, Apt1, NH_2-CTT ACG GTG GGG CAA TT, and Apt2, GTT TTG TTA CAG TTC GTT TCT TTT CCC TGT CTT GTT TTG TTG TCT-NH_2, were synthesized by Sangon Tech. Ltd. (Shanghai, China) without further purification. $AgNO_3$ (99.8%, Tongbai Xinhong Silver Products Co. Ltd, Henan, China), (3-Aminopropyl) triethoxysilane (APTES, 98%, Sigma-Aldrich), Tetraethyl orthosilcate (TEOS, ≥99%, Fluka), and 1-ethyl-3-(3-(dimethylamino) propyl)-carbodiimide (EDC, Sigma) were used as received. Coomassie brilliant blue R-250 was purchased from Beijing dingguo biotech CO. LTD. Other commercial reagents were analytical reagent grade without further purification. The solutions were prepared using ultrapure water, which was obtained through a Milli-Q Integral-5 water purification system (Millipore, U.S.A.) and had an electric resistance of 18.2 MΩ.

2.2. Apparatus

The plasmon resonance absorption (PRA) of AgNPs and Ag@SiO_2 was measured with a Hitachi U-3010 spectrophotometer (Tokyo, Japan). SEM observations were carried out on a Hitachi S-4800 scanning electron microscopy (Tokyo, Japan). A Zetasizer Nano-ZS System (Malvern Inc.) was used to detect the size of particles in solution based on photo correlation spectroscopy. The morphology of Ag@Si NPs in the absence and presence of prion protein was observed on Nanoscope Quadrex atom force microscope (AFM) (Veeco, USA), and Raman spectra were obtained on a LabRan HR800 Spectrometer (HORIBA Jobin Yvon, France) with 532 nm wavelength incident laser light.

2.3. Expression and purification of recombinant human prion protein

The plasmid rhPrP$_{23\text{-}231}$ was transformed to competent bacteria of strain *Escherichia coli* BL21-DE3 and induced by isopropyl-D-thiogalactopyranoside (IPTG). The cells were harvested and then purified according to the method developed by Xiao's group with some modifications [13–14,30]. Protein concentration was determined using the molar extinction coefficients $\varepsilon_{280}=56{,}590\ M^{-1}\ cm^{-1}$ for rhPrP (23–231) [31].

2.4. Synthesis and functionalization of Ag@Si NPs

Silver nanoparticles (AgNPs) were prepared according to the reference with small modification [32]. In short, 1.5 mL of 2% (w/w) trisodium citrate was added into a boiling 50.0 mL solution containing 1.0 mM $AgNO_3$ in a conical flask, followed by continuous stirring and boiling for about 20 min. For the synthesis and DNA-modified of Ag@Si NPs [33–35], 10 mL of the as-prepared silver colloid, which was then centrifuged at 500 rpm for 1 h to remove larger NPs, was transferred into a conical glass flask containing 40 mL of ethanol, followed by the addition of 0.8 mL of ~25% ammonia and 1.5 mL of 10 mM TEOS ethanol solution under vigorous shaking, respectively, and the resulting solution further react for 24 h at 30 °C. Ag@Si NPs were collected by centrifugation and further washed with ethanol three times. Subsequently, 0.2 mL of APTES was added to 4.8 mL of the as-obtained Ag@Si NPs and stirring at room temperature for 1 h, the reaction mixture was centrifuged to remove the unreacted APTES, and then heated to 120 °C for 1 h. Lastly, the aminated Ag@Si NPs were modified with NH_2-Apt according to the literature [33].

2.5. Protein labeling

A certain amount of PrP was mixed with 50 μL of 0.5 mg mL^{-1} R-250 (dissolved in PBS) and incubated for 10 min, and then, 50 μL of 1% BSA was added for blocking isolated R-250 molecules. After these additions, the solutions were well mixed.

2.6. General procedure

For the detection of PrP, Ag@Si-Apt1 were added to a certain amount of PrP/R-250 conjugates at 37 °C for 30 min, followed by the addition of Ag@Si-Apt2 and incubating at 37 °C for 1 h more in the presence of 20 mmol/L MES buffer (pH 6.25) and 0.25 mol/L NaCl. Ag@Si aggregates were collected and washed by

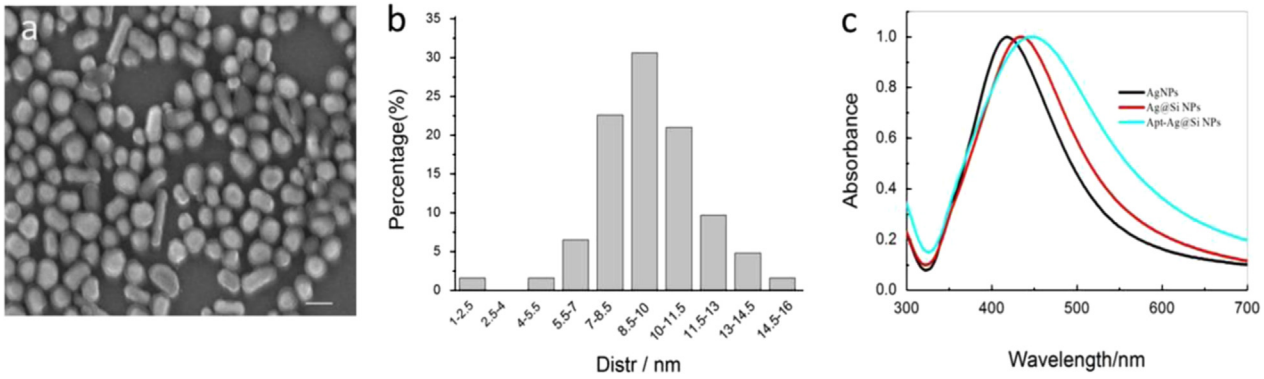

Fig. 1. Features of Si@Ag NPs during the modification process with aptamer. SEM images of Ag@Si NPs (a), scale bar, 400 nm. (b) Size information of silica shell. (c) Extinction spectra of AgNPs (black), Ag@Si NPs (red) and Ag@Si-Apt (cyan), respectively. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)