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Aptamers-based sandwich assay for silver-enhanced fluorescence multiplex detection

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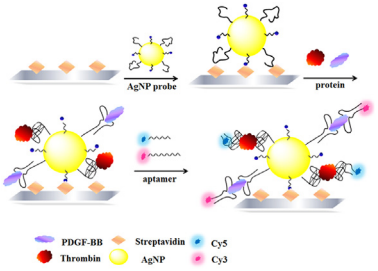
HIGHLIGHTS

- The dual aptamers substitute for antibodies.
- The silver-based microarray can enhance the sensitivity of proteins detection.
- The silver-based microarray can detect multiplex proteins simultaneously.
- The aptamer-based sandwich assay is sensitive and specific.

GRAPHICAL ABSTRACT



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ABSTRACT

In this work, aptamers-modified silver nanoparticles (AgNPs) were prepared as capture substrate, and fluorescent dyes-modified aptamers were synthesized as detection probes. The sandwich assay was based on dual aptamers, which was aimed to accomplish the highly sensitive detection of single protein and multiplex detection of proteins on one-spot. We found that aptamers-modified AgNPs based microarray was much superior to the aptamer based microarray in fluorescence detection of proteins. The result shows that the detection limit of the sandwich assay using AgNPs probes for thrombin or platelet-derived growth factor-BB (PDGF-BB) is 80 or 8 times lower than that of aptamers used directly. For multiplex detection of proteins, the detection limit was 625 pM for PDGF-BB and 21 pM for thrombin respectively. The sandwich assay based on dual aptamers and AgNPs was sensitive and specific.

1. Introduction

Protein microarray, as a high throughput analysis method for proteins, is increasingly becoming an important research tool for the study of proteins [1–3]. Fluorescence based detection is the most commonly used method on protein microarray, because it is sensitive, rapid, simple and comparatively inexpensive [4,5]. However, the traditional organic dyes labeling is often difficult to obtain a sufficient low detection limit for their limited quantum yields and low dye-to-reporter molecule labeling ratio. To meet the desired sensitivity, more and more attentions have been paid to the explosion of nanomaterials with unique optical properties, such as quantum dots, fluorescent silica nanoparticles and metallic nanomaterials [6–9]. The applications of them in fluorescence detection on the microarray have showed great advantages for the sensitive detection of proteins.

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Silver nanoparticles (AgNPs) are widely used in biological analysis for its excellent chemical and physical properties [10,11]. The applications of AgNPs are promising in the fluorescence detection for their metal enhanced fluorescence (MEF) effect [12–14]. Several researches have been made on the MEF effect of silver island film (SIF). The silver nanoparticles can be deposited on the glass slide to form SIF. The SIF can enhance fluorescence intensity of nearby dyes to accomplish the highly sensitive detection of DNA and proteins [15–18]. We have recently developed some novel fluorescence methods based on AgNPs, which show great potential in the biosensors and high throughput detection [19]. We have already showed the adenosine detection by using an aptamer-based AgNPs nanosensor [20], the multiple protein detection of IgE and thrombin using multicolor silver nanoprobes [21] and IgE and PDGF-BB detection by a plasmonic microarray [22]. Here they show a slight different application of AgNPs in aptamer-based sandwich assay for silver-enhanced fluorescence detection.

Aptamers are artificial, single-stranded DNA/RNA molecules, which are selected in vitro through systematic evolution of ligand by exponential enrichment (SELEX). Due to their high selectivity, chemical stability, versatile target binding and readily commercially available at low cost over traditional antibodies, aptamers are growing widely used as alternative affinity ligands for protein analysis [23–26]. So far, the majority of aptamer based methods are designed for a single target analysis [27]. Although some aptamer microarrays can achieve the multiplex detection of proteins [28,29], the cumbersome process of labeling proteins or the complicate detection protocols which are usually needed would limit their wide applications [30–32]. Therefore, developing new probes based on aptamers-modified nanomaterials are good ways to enhance detection sensitivity. We fabricated aptamers-modified AgNPs to enhanced the sensitivity of fluorescence detection and fulfill the multiplex detection of proteins.

In this report, a novel aptamers-modified AgNPs based sandwich assay is designed to detect proteins on microarray. Aptamers-modified AgNPs were first used as capture probes in the sandwich assay for sensitive fluorescence detection, in which dyes labeled secondary aptamers were used as report probes. We used this method to detect thrombin and PDGF-BB respectively. We found that compared with using aptamers as capture probes directly, the detection limits of using AgNP probes were decreased to 80 times and 8 times for thrombin and PDGF-BB respectively. Furthermore, two types of aptamers modified AgNPs were used as capture probes in the multiplex detection of thrombin and PDGF-BB on microarray to accomplish the simultaneous detection of proteins on one sample spot.

2. Experimental

2.1. Materials

All of the solutions were prepared with ultrapure water from a Millipore system. silver nitrate, ($AgNO_3$) and sodium borohydride ($NaBH_4$) were purchased from Sigma–Aldrich for preparing silver nanoparticles. 1 × PBS (NaCl 137 mmol/L, KCl 2.7 mmol/L, Na_2HPO_4 10 mmol/L, KH_2PO_4 2 mmol/L),1 × PBSM(1 × PBS + 1 mmol/L $MgCl_2$), 1 × PBST (1 × PBS + 0.05%Tween). We employed the following oligonucleotides in the design of aptasensor:

T_1: 5′-SH-AAAAAAAAAAAAAAAGGTTGGTGTGGTTGG-3′;
P_1:5′-SH-AAAAAAAA-TACTCAGGGCACTGCAAGCAATTGTGGTCCCAATGGGCTGAGTA-3′
C_{A15}: 5′-SH-AAAAAAAAAAAAAAA-bio-3′
T_2:5′-Cy5-AGTCCGTGGTAGGGCAGGTTGGGGTGACT-3′
P_2: 5′-Cy3-TACTCAGGGCACTGCAAGCAATTGTGGTCCCAATGGGCTGAGTA-3′
T_3:5′-bio-AAAAAAAAAAAAAAAGGTTGGTGTGGTTGG-3′
P_3:5′-bio-AAAAAAAA-TACTCAGGGCACTGCAAGCAATTGTGGTCCCAATGGGCTGAGTA-3′

(T_1 and T_3were for the primary thrombin aptamers, P_1 and P_3 were for the primary PDGF-BB aptamers, T_2 was for the secondary thrombin aptamer, P_2 was for the secondary PDGF-BB aptamer.) All the synthetic oligonucleotides used in this study were purchased from Shanghai Sangon Biotechnology Co. Thrombin was purchased from Sigma–Aldrich. PDGF-BB was purchased from R&D system (Minneapolis, MN).

2.2. Apparatus

A Luxscan-10K/A microarray Scanner (532 nm laser source for Cy3, 635 nm laser source for Cy5, Beijing Capital Bio Co. Ltd, China) was used for fluorescence imaging and quantitative detection and the images were analyzed through LuxScan3.0 software (CapitalBio). Scanning electron microscopy (SEM) and Transmission electron microscope (TEM) (JEM-200CX, Japan) were used for collecting SEM and TEM images, and CT15RT versatile refrigerated centrifuge from TECHCOMP(Shanghai, China) was used to centrifuge AgNPs.

2.3. ssDNA probe assembly at the surface of AgNPs

AgNPs was prepared by $NaBH_4$ reduction of $AgNO_3$ as previously reported [33]. For ssDNA probe assembly on the AgNPs, 1 mL of AgNPs solution was mixed with T_1 (50 μL, 10 μM) and C_{A15} (50 μL, 10 μM) to form conjugate 1 for the detection of thrombin, P_1 (50 μL, 10 μM) and C_{A15} (50 μL, 10 μM) to form conjugate 2 for the detection of PDGF-BB, or T_1 (25 μL, 10 μM), P_1 (25 μL, 10 μM) and C_{A15} (50 μL, 10 μM) to form conjugate 3 for multiplex detection, the mixture was incubated for at least 18 h at room temperature. Then, 122 μL of 1 × PBS was added to the solution to adjust the pH value and increase ionic strength of the resulting solution, and allowed to stand for 6 h. Three increments of 21 μL of 2 M NaCl were added at 3 h intervals in order to increase the NaCl concentration of the solution gradually. After standing for at least 48 h, the nanoparticles were isolated by centrifugation for 15 min, 14 °C, at 15000 rpm and washed with PBS (pH = 7.4) twice. The resulting silver-oligonucleotide conjugate was redispersed in PBSM (1 × PBS, 1 mM $MgCl_2$).

2.4. Analytical procedure

A 3 × 3 microarray was fabricated on aldehyde-modified slides by Smart Arrayer (3 × 6 = 18 reaction pond). First, 2.5 mg/mL of streptavidin was spotted on the slide and left to stand for 2 h at 37 °C and overnight in refrigerator. Then the slide was blocked by 10 mg/mL BSA for 1 h and washed with 1 × PBST for 3 times (5 min each time) and air dried. Secondly, 30 μL of AgNPs probes (conjugate 1 and conjugate 2 for single-color detection, conjugate 3 for multiple-color detection) were added into the reaction pool and allowed to react for 1 h at 37 °C, then the slides were washed as described before. Thirdly, 30 μL of different concentrations of thrombin, PDGF-BB (for the single-color detection) or the mixture of thrombin and PDGF-BB (for the multiple-color detection) were added and allowed to react for 1 h at 37 °C followed by the washing step as described before. Finally, T_2, P_2 or the mixture of them was added to bind with target proteins at 37 °C for 1 h then subjected to the same washing step as before. The array was scanned and the data were collected by Luxscan-10K/A microarray Scanner.