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A non-aggregation colorimetric assay for thrombin based on catalytic properties of silver nanoparticles

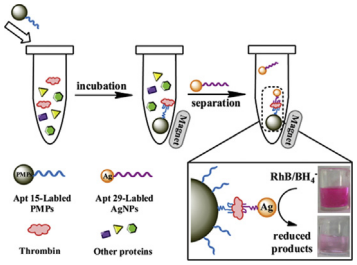
Jie Li, Wei Li, Weibing Qiang, Xi Wang, Hui Li, Danke Xu*

State Key Laboratory of Analytical Chemistry for Life Science, School of Chemistry and Chemical Engineering, Nanjing University, Nanjing 210093, China

HIGHLIGHTS

- An AgNP-based non-aggregation colorimetric aptasensor was first developed.
- The colorimetric principle was based on AgNP-catalyzed reductive degradation of RhB.
- This assay combined magnetic separation with nanocatalytic amplification.
- The detection limit of thrombin was as low as 0.2 nM with excellent specificity.

GRAPHICAL ABSTRACT



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ABSTRACT

In this paper, we developed a simple and rapid colorimetric assay for protein detection based on the reduction of dye molecules catalyzed by silver nanoparticles (AgNPs). Aptamer-modified magnetic particles and aptamer-functionalized AgNPs were employed as capture and detection probes, respectively. Introduction of thrombin as target protein could form a sandwich-type complex involving catalytically active AgNPs, whose catalytic activity was monitored on the catalytic reduction of rhodamine B (RhB) by sodium borohydride ($NaBH_4$). The amount of immobilized AgNPs on the complex increased along with the increase of the thrombin concentration, thus the detection of thrombin was achieved via recording the decrease in absorbance corresponding to RhB. This method has adopted several advantages from the key factors involved, i.e., the sandwich binding of affinity aptamers contributed to the increased specificity; magnetic particles could result in rapid capture and separation processes; the conjugation of AgNPs would lead to a clear visual detection. It allows for the detection limit of thrombin down to picomolar level by the naked eye, with remarkable selectivity over other proteins. Moreover, it is possible to apply this method to the other targets with two binding sites as well.

1. Introduction

It is of great importance to develop new methodologies for specific and sensitive protein detection in many biological and medical diagnostic fields. The advances of nanomaterials have provided exciting technologies and novel materials for protein detection based on the unique properties associated with nanoscale phenomena such as plasmon resonance, catalysis and energy transfer [1]. Among various methods, simple colorimetric methods have attracted significant consideration due to its rapidness and simplicity without complicated analytical instruments [2]. In addition, they allow direct and on-site visualization analysis for biological samples by the naked eye. The nanomaterial-based colorimetric mechanism is generally dependent on their inherent optical or catalytic properties. For example, gold nanoparticles (AuNPs) serve as exciting colorimetric reporters, relying on the easily visualized

* Corresponding author at: State Key Laboratory of Analytical Chemistry for Life Science, School of Chemistry and Chemical Engineering, Nanjing University, 22 Hankou Road, Nanjing 210093, China. Tel.: +86 25 83595835; fax: +86 25 83595835.
E-mail address: xudanke@nju.edu.cn (D. Xu).

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colour change in response to the surface plasmon resonance (SPR) absorption between well-spaced individual AuNPs (red) and aggregated AuNPs (blue) [3–9]. In contrary to AuNPs, silver nanoparticles (AgNPs) exhibit higher extinction coefficients than AuNPs at the same size. A collection of AgNP-based aggregation colorimetric assays have also been constructed [10–13]. However, the aggregation-based methods usually suffer from limitations of relatively low sensitivity, which is mainly caused by the lack of amplification of the detection signal [14].

Recently, the intrinsic enzyme-like activity of nanoparticles has become a growing area of interest in colorimetric assays [15,16]. These nanomaterial-based artificial enzymes (nanozymes), such as Fe_3O_4 magnetic nanoparticles have been established as highly stable and low-cost alternatives to natural enzymes, which could directly catalyze the oxidation of the corresponding substrates to achieve visual detections [17–19]. Nevertheless, nanozymes have inevitable disadvantages like low efficiency, low selection, and limited types of catalytic reactions, which might restrict their widespread applications [20].

In our previous work, a series of bioassays making full use of metal-enhanced fluorescence (MEF) effect of silver nanostructure have been developed [21–23]. We have gained rich experience in preparation and biofunctionalization of nanosilver. As one of the most fascinating nanomaterials, AgNPs are proved to be effective catalytic materials for various applications because of their large surface-to-volume ratio and electronic properties [24–30]. AgNPs as nanocatalysts have more active sites on their surface than enzymes, which could effectively catalyze specific substrates to produce colorimetric signals [31,32]. Furthermore, they facilitate electron transfer more efficiently and cost much lower than other noble metals (Au, Pd and Pt). To the best of our knowledge, catalytic properties of AgNPs have never been reported in the colorimetric sensing detection of proteins.

Thrombin is a key protein that catalyzes many coagulation-related reactions responsible for blood clotting [33,34]. In this report, thrombin was used as a model analyte and proof-of-concept experiments could be performed. The well-known DNA aptamers that could recognize two different epitopes of the protein were employed as bridge linkers in a sandwich-type complex. The magnetic particles were modified with the aptamers to collect target protein from the sample matrix via a magnetic field; such rapid separation process could be applied for protein detection in biological samples. Inspired by the facts mentioned above, a sandwich-like aptasensor was developed incorporating magnetic particles for protein separation and enrichment, as well as catalytically active AgNPs for colorimetric detection. The typical $NaBH_4$-mediated catalytic reduction of rhodamine B (RhB) was performed for the visual observation and optical measurement by UV–vis spectroscopy. The results indicated that the target protein could be detected with high sensitivity and excellent specificity using the suggested method.

2. Experimental

2.1. Materials

Oligonucleotides used in this study were synthesized and purified by Sangon Biotech Co. LLC (Shanghai, China) with following sequences. The biotinylated 15-mer anti-thrombin aptamer (denoted as Apt 15): 5′-biotin-A_{15}-GGT TGG TGT GGT TGG-3′. The thiolated 29-mer anti-thrombin aptamer (denoted as Apt 29): 5′-SH-A_{15}-AGT CCG TGG TAG GGC AGG TTG GGG TGA CT-3′. Human serum albumin (HSA), immunoglobulin A (IgA), immunoglobulin G (IgG), lysozyme and bull serum albumin (BSA) were purchased from Biosharp (Japan). Human α-thrombin, rhodamine B (RhB), silver nitrate, and sodium borohydride ($NaBH_4$) were ordered from Sigma–Aldrich Co. LLC (USA). Streptavidin coated paramagnetic particles (PMPs ca. 1.0 μm) were provided by Promega Co. (Madison, WI, USA).

Buffers used in this work: 1× PBS (137 mM NaCl, 2.7 mM KCl, 10 mM $Na_2HPO_4{\cdot}12H_2O$, 2 mM KH_2PO_4, pH 7.4), 0.5× SSC (75 mM NaCl, 7.5 mM trisodium citrate, pH 7.0), buffer A (50 mM tris–HCl, 2 M NaCl, 0.1% tween 20, pH 7.4), buffer B (20 mM tris–HCl, 140 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, 1 mM $CaCl_2$, pH 7.4), protein-free blocking solution (1% content in tris-buffered salines, pH 7.4), stock solution (buffer B containing 0.1% BSA).

All the chemicals were of analytical grade. Ultrapure water (≥18.20 MΩ) used throughout the experiments were generated by a millipore water purification system.

2.2. Apparatus

The UV–vis absorption spectra were recorded with a Nanodrop 2000c spectrophotometer (Thermo Scientific, USA). Measurement of the absorbance and absorption spectra of rest dye after reduction was conducted on a Synergy NEO HTS multi-mode microplate reader (Biotek, USA).

2.3. Preparation of AgNPs and Apt 29-AgNPs

AgNPs with an average diameter of 20 nm were synthesized based on a previously described protocol with some modifications [22]. Briefly, ice cold $AgNO_3$ (2 mM) was added dropwise to twice the volume of $NaBH_4$ (3 mM) with vigorous stir in an ice-water bath. Then the solution was transferred into a hot bath. After being cooled down to room temperature with continuous stir, the prepared AgNPs were stored at 4 °C.

The Apt 29-AgNPs were synthesized as follows. 1 mL solution of as-prepared AgNPs was mixed and incubated with Apt 29 (1 nmol) for 16 h at room temperature. Then the mixture was aged in salt and brought to a final concentration of 0.1 M NaCl through a stepwise process. Followed by incubation for 40 h, the solution was centrifuged at 15,000 rpm for 20 min to remove unbound aptamers. The precipitate was washed three times with tris–HCl (10 mM, pH 7.4) and finally dispersed in stock solution at 4 °C for further use.

2.4. Preparation of Apt 15-PMPs

Before modification, paramagnetic particles (PMPs) coated with streptavidin were pretreated by washing twice with 0.5× SSC and buffer A, respectively. Then magnetically collected PMPs were redispersed in 300 μL of buffer A and mixed with certain amount of Apt 15 (at a ratio of 2 nmol aptamer to 1 mg PMPs). The mixture was incubated for 1 h at 37 °C with gentle shaking. Subsequently, the Apt 15-PMPs were rinsed with buffer B containing 0.1% Tween twice and dispersed in 600 μL of blocking solution for 2 h in order to block the nonspecific binding sites. After the washing steps, the Apt 15-PMPs was kept in 600 μL of stock solution at 4 °C until use, with a final concentration of about 1 mg mL^{-1}.

2.5. Fabrication of Apt 15-PMPs/thrombin/Apt 29-AgNPs sandwich complex

20 μL of Apt 15-PMPs and 200 μL of protein solution with a series of concentration were mixed and incubated for 2 h at 37 °C with gentle shaking. Followed by adding 100 μL of Apt 29-AgNPs into the above solution, the mixture was reacted for another 1.5 h to form a sandwich complex.

After repetitive washing and separation procedures, unbound AgNPs were removed, and the final sandwich complex was suspended in 40 μL of buffer B for the following colorimetric detection.