Analytica Chimica Acta 828 (2014) 92–98

Contents lists available at ScienceDirect

Analytica Chimica Acta

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

Label-free detection of adenosine based on fluorescence resonance energy transfer between fluorescent silica nanoparticles and unmodified gold nanoparticles

Weibing Qiang, Haiping Liu, Wei Li, Xiang Chen, Danke Xu *

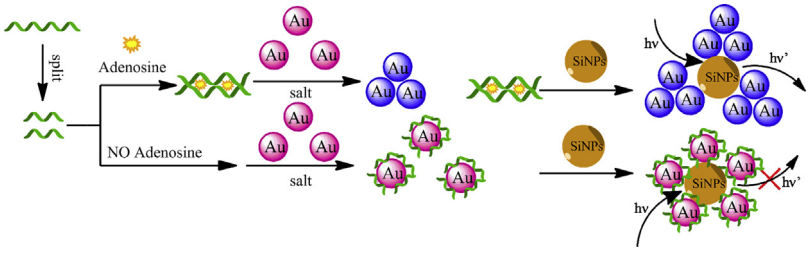
State Key Laboratory of Analytical Chemistry for Life Science, School of Chemistry and Chemical Engineering, Nanjing University, Nanjing, 210093, China

HIGHLIGHTS

- A novel assay method based on FRET between fluorescent SiNPs and AuNPs was developed.
- The method was based on the conformation change of aptamer lead by target recognition.
- The aptamer toward adenosine without modification was split into two fragments.
- The method was label-free without the modification of fluorescent SiNPs or AuNPs.
- The detection limit of adenosine was as low as 45 nM with excellent specificity.

GRAPHICAL ABSTRACT

?
?
?
?



ARTICLE INFO

Article history:
Received 17 January 2014
Received in revised form 10 April 2014
Accepted 19 April 2014
Available online 26 April 2014

Keywords:
Aptamer
Fluorescent silica nanoparticles
Gold nanoparticles
Fluorescence resonance energy transfer
Adenosine

ABSTRACT

A sensitive and convenient strategy was developed for label-free assay of adenosine. The strategy adapted the fluorescence resonance energy transfer property between Rhodamine B doped fluorescent silica nanoparticles (SiNPs) and gold nanoparticles (AuNPs) to generate signal. The different affinities of AuNPs toward the unfolded and folded aptamers were employed for the signal transfer in the system. In the presence of adenosine, the split aptamer fragments react with adenosine to form a structured complex. The folded aptamer cannot be adsorbed on the surface of AuNPs, which induces the aggregation of AuNPs under high ionic concentration conditions, and the aggregation of AuNPs leads to the decrease of the quenching ability. Therefore, the fluorescence intensity of Rhodamine B doped fluorescent SiNPs increased along with the concentration of adenosine. Because of the highly specific recognition ability of the aptamer toward adenosine and the strong quenching ability of AuNPs, the proposed strategy demonstrated good selectivity and high sensitivity for the detection of adenosine. Under the optimum conditions in the experiments, a linear range from 98 nM to 100 μM was obtained with a detection limit of 45 nM. As this strategy is convenient, practical and sensitive, it will provide a promising potential for label-free aptamer-based protein detection.

* 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./fax: +86 25 83595835.
E-mail addresses: qiangwb1987@126.com (W. Qiang), yoyo563727570@163.com (H. Liu), weilixj8510@163.com (W. Li), xchen1025@nju.edu.cn (X. Chen), xudanke@nju.edu.cn (D. Xu).

http://dx.doi.org/10.1016/j.aca.2014.04.043
0003-2670/

1. Introduction

Aptamers, obtained from random-sequence nucleic acid libraries by an *in vitro* evolution process called SELEX (systematic evolution of ligands by exponential enrichment), are single-strand DNA/RNA oligonucleotides with high specificity and affinity against many given targets ranging from small inorganic and organic molecules to macromolecules and even cells [1–3]. Upon binding with the targets, aptamers often undergo significant conformational changes into hairpin, stem-loop, G-quadruplex, pseudoknot or bulge structures [4]. The aptamers have many merits, such as easy storage, excellent chemical stability and reproducibility, and wide applicability [5,6]. Recently, a variety of aptamer-based analytical methods have been developed for molecular recognition and detection, including electrochemistry [7–9], fluorescence [10–12], SPR [13], quartz crystal microbalance [14] *etc.*

Due to some distinct advantages, dye-doped silica nanoparticles, which use silica as the shell and fluorophores as the core, have attracted more and more attentions [15–18]. The entrapment of the organic molecules within the silica matrix results in an increase of the quantum yield of the fluorophores, thus enhancing the overall brightness of the fluorescent probe. Therefore, the use of SiNPs as labeling reagents for biomolecular assay can provide higher sensitivity than dye molecules [19,20]. The silica matrix serves as a protective shell, reducing the impact of the surrounding environment on the fluorescent dyes core. Thus, the fluorescent SiNPs are anti-photobleaching and have excellent photostability. More importantly, unlike metal nanoparticles and quantum dots, the entrapment enhances higher hydrophilicity, biocompatibility, and stability of the fluorophores under physiological conditions. The nanoparticles exhibit little or no cytotoxicity, which makes them be promising for *in vivo* observation of cell trafficking, tumor targeting, and disease diagnosis and treatment [21–23].

Because of their optical and electronic properties, gold nanoparticles (AuNPs) have attracted significant research attention in numerous fields, including catalysis, diagnostics and biosensors [24–28]. Specifically, on the basis of their excellent extinction coefficients and strongly distance-dependent optical properties, AuNPs have been successfully employed as colorimetric indicators in both chemical and biological detections [29–31]. In these studies, AuNPs modified with thiolated DNA probes were extensively adopted. The interaction between targets and DNA probes led to a change in the color of AuNPs solution from red to blue. Noticeable, the modification of AuNPs with thiolated DNA is a rather time-consuming process. Therefore, unmodified AuNPs are more preferable colorimetric probes in current research [32–35]. Under high ionic concentration condition, the electrostatic repulsion between negatively charged AuNPs is screened, resulting in the aggregation of AuNPs. It has been proved that nonthiolated short single-strand DNA can be adsorbed onto AuNPs surface, and will avoid AuNPs aggregation [36]. On contrary, folded DNA cannot bind with AuNPs effectively, which makes AuNPs susceptible to salt-induced aggregation. The process of AuNPs aggregation also results in a red-to-blue color change due to the interparticle coupled plasmon excitons in the aggregated states. However, the colorimetric methods based on unmodified AuNPs have the disadvantage of low sensitivity. Apart from these properties, accumulating experimental evidence suggests that AuNPs can effectively quench fluorescence of organic dyes and quantum dots, which is probably because of nonradiative energy transfer from the fluorophore to the metal [37–40]. Considering the high quenching efficiency of AuNPs to almost all fluorophores, lots of nanosensors for biomolecule detection have been developed without optimization of fluorophore-quencher pairs [41–44].

In this study, we combined the advantages of label-free aptamers, unmodified AuNPs and dye-doped fluorescent SiNPs to develop a sensitive strategy for label-free assay of adenosine. The fluorescence of the Rhodamine B doped fluorescent SiNPs was quenched by aptamer-wrapped AuNPs. The introduction of adenosine in the system would lead to a change in aptamer conformation, which would further induce the aggregation of AuNPs. The quenching ability of AuNPs would be decreased by the aggregation, and the fluorescence of the fluorescent SiNPs was restored. The proposed biosensor system has exhibited satisfied selectivity and high sensitivity, and holds great potentialities in clinical applications.

2. Materials and methods

2.1. Chemicals and apparatus

Rhodamine B isothiocyanate (RITC), (3-aminopropyl) triethoxysilane (APTS), adenosine, cytidine, uridine and guanosine were purchased from Sigma–Aldrich, Co. LLC., USA. All the oligonucleotides used in this work were synthesized and purified by Sangon Biotechnology Co., Ltd. (Shanghai, China) with the following sequences. The 27-mer DNA aptamer (Apt-27) against adenosine: 5′-ACC TGG GGG AGT ATT GCG GAG GAA GGT-3′. The split aptamer fragments Apt-14: 5′-ACC TGG GGG AGT AT-3′ and Apt-13: 5′-TGC GGA GGA AGG T-3′. A 15-mer oligonucleotide with randomly chosen irrelevant sequence Oligo: 5′-GGT TGG TGT GGT TGG-3′. Tris–HCl buffer was purchased from Sangon. Tetraethyl orthosilicate (TEOS), chloroauric acid and trisodium citrate were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). All other reagents were of analytical reagent grade and used without further purification. Ultrapure water was used throughout the experiments. Synergy NEO HTS multi-mode microplate reader (Biotek, USA) was employed to detect the absorption spectra of gold nanoparticles and the fluorescence emission spectra of fluorescent silica nanoparticles. Scanning electron microscopy (SEM) (S-4800, Japan) was used for collecting SEM images.

2.2. Preparation of gold nanoparticles

Gold nanoparticles (AuNPs) were synthesized by thermal reduction of $HAuCl_4$ with sodium citrate. Briefly, 4 mL of 1% trisodium citrate solution was added to a boiling 1% $HAuCl_4$ solution with rapidly stirring. The solution was kept boiling and stirred for 20 min. After being cooled down to room temperature, the prepared AuNPs were stored at 4 °C. The obtained spherical AuNPs have an average diameter of 13.30 ± 1.63 nm, with a maximum absorbance at 520 nm (Fig. 1A, C) The zeta potential of the AuNPs was −34.0 mV.

2.3. Preparation of fluorescent silica nanoparticles

Fluorescent Rhodamine B doped core-shell silica nanoparticles (SiNPs) were synthesized by the Stöber method. Briefly, RITC (5.36 mg, 0.01 mol) was dissolved in distilled ethanol (800 μL), and APTS (13.6 μL, 0.08 mmol) was added with stirring. The APTS was then allowed to react with the dye for 20 h in the dark. Aqueous ammonia solution (25%, 2 mL) was added along with ethanol (30 mL) and the mixture was stirred at room temperature for 20 h. TEOS (0.558 mL, 2.5 mmol) was added to the mixture, and the reaction was allowed to proceed for another 24 h in the dark with continuous stirring. TEOS (13.8 μL, 0.062 mmol) was added last and the mixture was stirred for another 5 h. The resultant suspension was centrifuged at 15,000 rpm for 10 min to remove the remaining reagents in supernatant. The particles were washed with 70% ethanol and water. The SiNPs were stored at room temperature after drying. In this case, uniform, spherical SiNPs in the nanometer range were obtained with a diameter of 69.29 ± 4.07 nm (Fig. 1B). And the SiNPs had a high