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A colorimetric aptamer biosensor based on cationic polymer and gold nanoparticles for the ultrasensitive detection of thrombin

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ABSTRACT

A colorimetric assay for the ultrasensitive determination of thrombin based on cationic polymer and gold nanoparticles was presented, in which unmodified gold nanoparticles (AuNPs) was used as probes and 21-mer thrombin-binding aptamer (TBA) as sensing elements. Upon the addition of thrombin, TBA interacted specifically with thrombin to form a G-quadruplex structure. As a result, the conformation change facilitated the cationic polymer, poly(diallyldimethylammonium chloride) (PDDA)-induced AuNP aggregation. Thus, the visible change in color from wine-red to blue–purple was readily seen by the naked eye. The colorimetric sensor could detect thrombin down to 1 pM with high selectivity in the presence of other interferring proteins. Furthermore, the assay was successfully employed to determine thrombin in human serum sample, which suggested its great potential for diagnostic purposes.

1. Introduction

Thrombin is an important multifunctional enzyme involved in many physiological and pathological processes, such as blood coagulation, thrombosis, inflammation, angiogenesis, tumor growth and metastasis (Zhao and Gao, 2013). Thrombin can be used as a therapeutic and a biomarker for diagnosis of some diseases, such as pulmonary metastases and diseases associated with coagulation abnormalities (Licari and Kovacic, 2009; Kitamoto et al., 2008). Under normal conditions, the concentration of thrombin in blood varies from nanomolar to low micromolar levels during the coagulation progress (Arai et al., 2006). The current clinical methods for protein detection rely heavily on antibodies (Shuman and Majerus, 1976; Bichler et al., 1991; Zhu et al., 2000). Although these conventional strategies provide accurate and sensitive detection of proteins, there are still some inconveniences that exist, such as the utilization of radioactive substances, enzyme labeling, time-consuming processes, and technical expertise as well as sophisticated equipment. Thus, development of protein sensing methods that are rapid, simple, sensitive, selective, on-site and cost-effective is still highly desirable.

Aptamers are single stranded DNA molecules or RNA that selectively bind to various target molecules with high affinity, such as small molecules, proteins and drugs (Zuo et al., 2007; Lai et al., 2007; Bang et al., 2013). Compared to traditional molecular recognition system, aptamers have a lot of advantages in terms of the simplicity of synthesis. Aptamers can be stored stable for a long term, ease of labeling, excellent stability, wide applicability, and high sensitivity. As a consequence, their properties make them very attractive for applications in medical diagnosis, environmental monitoring and biological analysis (Li et al., 2010; Song et al., 2008; Zhang et al., 2008; Wang et al., 2009). Different detection techniques such as optical (Chang et al., 2010; Huang et al., 2010; Yan et al., 2011), electrochemical (Bang et al., 2005; He et al., 2007; Xiao et al., 2005; Zhao et al., 2011), surface enhanced resonance Raman scattering (SERRS) (Cho et al., 2008), electrochemiluminescence (Wang et al., 2011), surface plasmon resonance (Ostatna et al., 2008; Polonschii et al., 2010) and so on have been developed to detect thrombin. However, they are limited by the sensitivity, in which only nanomolar or micromolar concentrations of thrombin are detectable. Compared with those sophisticated equipment, colorimetric sensors gained increasing attention for its very short assay time (merely several minutes), relatively low cost and no requirement for skillful technicians (Han and Kim, 2002). Until now, colorimetric aptasensor for thrombin detection has rarely been reported.

Herein, we developed a simple and ultrasensive colorimetric aptasensor for thrombin detection based on AuNPs and water-soluble cationic polymer (PDDA) instead of salt with a high concentration, because the sensors based on salt-induced AuNP aggregation suffer from relatively higher detection limits (Yang et al., 2011; Zheng et al., 2011; Song et al., 2011). Otherwise, PDDA exhibits not only a significant advantage in relation to the aggregation of AuNPs but also electrostatic interactions with DNA

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(Ofir et al., 2008; Peng et al., 2006; Xia et al., 2010). Using these properties, we developed a colorimetric aptamer biosensor for thrombin detection based on PDDA and TBA-mediated aggregation of AuNPs. The results indicated that the method was simple, cheap, and highly sensitive and selective for thrombin detection and could be successfully used in the quantitative detection of thrombin in human serum samples.

2. Experimental

2.1. Reagents and apparatus

TBA oligonucleotides (5′-TTTGGTTGGTGTGGTTGGTTT-3′) were synthesized by Sangon Biotechnology Co. Ltd. (Shanghai, China). Sodium tetrachloroaurate(III) ($HAuCl_4$), sodium citrate and tris-base were purchased from Sigma-Aldrich (USA). PDDA was obtained from Aladdin reagent (Shanghai) Co. Ltd. All other reagents are of analytical reagent grade. All solutions were prepared with Tris–HCl buffer solution (pH 7.41).

Ultraviolet–visible (UV–vis) absorption spectra were recorded on an UV-2550 Spectrophotometer (Shimadzu Corporation). Transmission electron microscope (TEM) observations were carried out with a JEOL JEM2010 microscope at 200 kV.

2.2. Preparation of AuNPs

The 15-nm diameter AuNPs were prepared by the citrate-mediated reduction of $HAuCl_4$ according to the published protocol (Storhoff et al., 1998). The obtained AuNP solution (4.9 nM) was cooled to room temperature and stored at 4 °C. The concentration of these AuNP solution was determined by UV–vis spectroscopy using an extinction coefficient of $2.7\times10^8\ M^{-1}\ cm^{-1}$ at λ=520 nm for 15 nm AuNPs (Jin et al., 2003).

2.3. General procedure of colorimetric sensing of thrombin

First, 498 μL of thrombin with various concentrations were respectively added into a 1.5 mL plastic vial containing 1 μL TBA solution (10 μM) in 20 mM Tris–HCl buffer solution (pH 7.41). After incubation for 20 min at 37 °C, 1 μL of PDDA solution (10 μM) was added. Reacting for 10 min, then 500 μL of AuNP solution (4.9 nM) was added quickly into this vial and mixed thoroughly. After another 5 min of incubation, 1000 μL of the resulting solution was transferred to a 1 cm micro quartz cuvette for spectral recording. In addition to incubation of thrombin at 37 °C, all other assays were performed at room temperature.

3. Results and discussion

3.1. Principle of colorimetric method for thrombin detection

A schematic representation of the mechanism of the colorimetric sensing thrombin is illustrated in Fig. 1. In the absence of target thrombin, free single-stranded DNA (ssDNA) with a random coil structure interacted with cationic polymer, PDDA through electrostatic interaction. Aptamer and PDDA formed a duplex structure (Wu et al., 2012). Thus PDDA was not sufficient to induce the aggregation of AuNPs. However, upon the addition of thrombin, the aptamer with a random coil structure could be changed into a G-quadruplex structure. Therefore, the remaining PDDA could link the AuNPs together to make the aggregation. The aggregation of AuNPs led to a change in color from wine-red to blue–purple.

3.2. Optimization of experimental conditions

To optimize the sensing conditions, various concentrations of PDDA (1, 3, 5. 8, 10, 30 and 50 nM) were added into a micro cuvette containing 500 μL Tris–HCl buffer solution (20 mM, pH 7.41), then

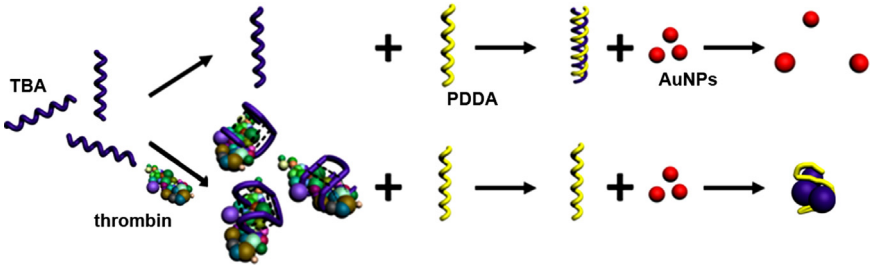

Fig. 1. Schematic representation of the sensing procedure for colorimetric detection of thrombin based on cationic polymer and gold nanoparticles.

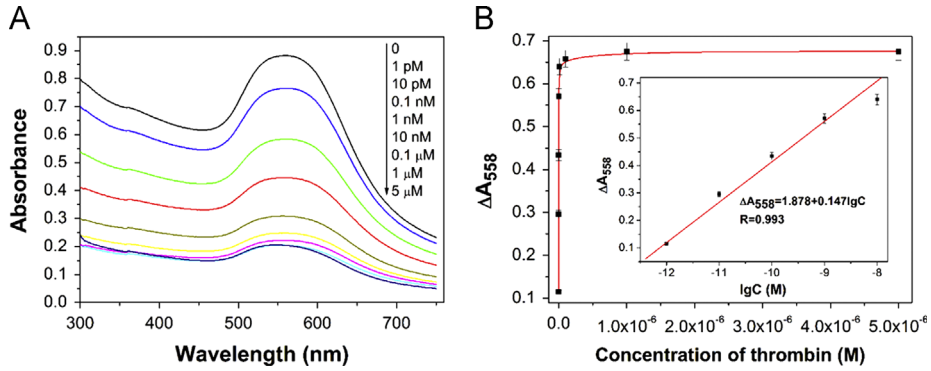

Fig. 2. (A) The UV–vis absorbance spectra of AuNPs in the presence of 10 nM PDDA and 10 nM TBA in 20 mM Tris–HCl buffer solution (pH 7.41) containing different concentrations of Thrombin (1 pM–5 μM). (B) The peak absorbance change at 558 nm as a function of thrombin concentration. Inset: the peak absorbance change of 558 nm is linear with logarithm of thrombin concentration over the range from 1 pM to 10 nM.