Biosensors and Bioelectronics 70 (2015) 372–375

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

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

Short communication

Up-conversion fluorescence "off-on" switch based on heterogeneous core-satellite assembly for thrombin detection

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ARTICLE INFO

Article history:
Received 11 February 2015
Received in revised form
21 March 2015
Accepted 26 March 2015
Available online 27 March 2015

Keywords:
Up-conversion fluorescence
Core-satellites
Thrombin
Aptamer sensor
Detection

ABSTRACT

NaGdF4: Yb, Er nanoparticles, with up-conversion (UC) fluorescence, were used for the first time to build an "off-on" switch based on Au core-UC satellites for thrombin detection. We fabricated the fluorescence sensor using thrombin aptamer modified Au core and complementary sequence modified UC satellites in liquid phase. With optimized assembled conditions, the yield of Au core-UC satellites achieved 80%. The fluorescence of UC nanoparticles quenched when satellite NP attached to Au core NP. Thrombin aptamer on the surface of Au core would bind to targets when thrombin existed in the system, then UC satellites were released and the quenched fluorescence recovered. The sensor showed high specificity for thrombin compared with other biomolecules and the limit of detection reached 3.5 fg/mL. Application of this sensor to detect targets in human serum also achieved satisfactory results. The purpose of this work was to build an ultrasensitive sensor based on Au core-UC satellites for thrombin detection in human serum to achieve diagnosis of diseases.

1. Introduction

Thrombin, generated in plasma by the conversion of its precursor, prothrombin, is involved in thrombosis and platelet activation, and plays a significant role in a number of cardiovascular diseases such as cerebral ischemia and infarction (Centi et al., 2007). Thrombin usually leads to vasospasm following subarachnoid hemorrhage. Blood flowing out from ruptured cerebral aneurysm clots around a cerebral artery, releasing thrombin. This can induce an acute and prolonged narrowing of the blood vessel, which might cause cerebral ischemia and infarction (stroke). Thrombin is not present in the blood of healthy individuals, but can be detected in the blood of patients suffering from diseases associated with coagulation abnormalities (Centi et al., 2007; Tennico et al., 2010). Therefore, the detection of thrombin is vital for the diagnosis of related diseases.

An aptamer is a single oligonucleotide which is completely engineered in an in vitro selection process, and has high affinity for targets and good stability and storage properties compared to antibodies (Cho et al., 2008). Since the thrombin-binding aptamer (15-mer, 5′-GGTTGGTGTGGTTGG-3′) was selected (Daniel et al., 2013; Li et al., 2008), numerous aptasensors (Chen et al., 2010b; Pavlov et al., 2004; Wang and Liu, 2009; Xiao et al., 2005) have been fabricated based on the conformational changes induced by target binding, signals used for quantification ranged from color (Chen et al., 2010a; Chen et al., 2014b; Li et al., 2008; Pavlov et al., 2004), fluorescence (Chang et al., 2010; Chi et al., 2011; Jie and Yuan, 2012; Kim and Lee, 2014; Kong et al., 2013; Wang et al., 2011b; Yan et al., 2011) and electrochemiluminescence (Chen et al., 2014a; Deng et al., 2014; Shan et al., 2011; Wang et al., 2011a) to surface enhanced Raman scattering (SERS) (Z. Wu et al., 2013) and surface plasmon resonance (SPR) (Bai et al., 2013; He et al., 2014; Mani et al., 2011). These methods have the disadvantages of either a high limit of detection or complex operation procedures and limit their application in blood samples (Cho et al., 2008).

The aim of this study was to design a fluorescence aptasensor with high sensitivity and the ability to resist interference in a matrix such as human serum. The sensor was based on the signal produced by the assembly and disassembly of gold core and up-conversion nanoparticle (UC NP) satellites. Compared with normal fluorescent materials, the fluorescence and chemical properties of UC NPs were more stable, the excitation of 980 nm can avoid the interference of auto fluorescence of biomolecules, such as protein (Wen et al., 2013; H. Wu et al., 2013; Zhang et al., 2014). The low biological toxicity of this sensor also demonstrated significant potential in biological applications such as cancer diagnosis and treatment (Cheng et al., 2013; Li et al., 2013, 2014; Maji et al., 2014).

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http://dx.doi.org/10.1016/j.bios.2015.03.068
0956-5663/

2. Material and methods

2.1. Materials and regents

The thiol-modified oligonucleotides (thrombin aptamer (TB-Apt) and its complementary sequence (TB-Apt C)) (purity $>95\%$) used in this study were manufactured by Shanghai Sangon Biological Engineering Technology & Services Co., Ltd., China. These oligonucleotides were dissolved in TE buffer (10 mM Tris–hydrochloride buffer, pH 8.0, containing 1.0 mM EDTA, Shanghai Sangon) to give a final concentration of 100 μM. Human α-thrombin (TB), bovine serum albumin (BSA), human prostate-specific antigen (PSA), serum albumin (HSA), immunoglobulin G (IgG), and L-cysteine (L-cys) were purchased from Sigma-Aldrich. Up-conversion nanoparticles (UC NPs) were purchased from Beijing Oneder-Hightech Co. Ltd., China. Deionized (DI) water, obtained using a Milli-Q device (18.2 MΩ, Millipore, Molsheim, France), was used throughout this work.

The detailed sequences of the oligonucleotides are as follows:

TB-Apt: 5′-HS-TTTTTGGTTGGTGTGGTTGG-3′
TB- Apt C: 5′-HS-TTTTTTTAATTATATTAACC-3′

2.2. Instrumentation

Fluorescence spectra were acquired using a F-7000 fluorescence spectrophotometer, scan speed was 240 nm/min. Transmission electron microscopy images were acquired using a JEOL JEM-2100 operating at an acceleration voltage of 200 kV.

2.3. Synthesis of gold nanoparticles

Gold nanoparticles (32 ± 1 nm) were synthesized by a seed-mediated growth method. Seeds (Au NPs of 13 ± 1 nm) were synthesized by a routine method: 2 mL of 38.8 mM trisodium citrate was quickly added to a boiling solution of $HAuCl_4$ (40 mL, 0.5 mM) with vigorous stirring and refluxed until there were no more color changes in the solution. Then, 7.5 mL of 5.3 mM ascorbic acid was injected into the mixed solution (2 mL–10 mM $HAuCl_4$, 0.1 mL–10 mM $AgNO_3$, 42.5 mL H_2O, 4 mL seeds) with a constant-flow pump at a speed of 0.6 mL/min under vigorous stirring. A BPS (bis(p-sulfonatophenyl) phenylphosphine dihydrate, dipotassium salt) solution was added to the as-synthesized Au NPs to a final concentration of 2.5 mg/mL and stirred in darkness for 12 h. TEM image of Au NPs was shown in Fig. S1a.

2.4. Oligonucleotide-functionalization of AuNPs and UC NPs

Au NPs were modified with thiolated TB-Apt using a previously reported method (Rosi et al., 2006). AuNPs (32 ± 1 nm) were suspended in 10 mM PB (pH 7.2) and 0.01% Tween20. TB-Apt (dissolved in TE buffer) was added to the AuNPs at a final ratio of 500 oligonucleotides per particle. The mixed solution was left for 2 h, a NaCl solution of 5 M in water was added (1 μL NaCl solution to 100 μL solution) with an interval of 30 min to bring the final concentration of NaCl to 400 mM at room temperature. The TB-Apt-modified AuNPs were washed three times with 10 mM PB buffer.

The ligand of UC NPs purchased from Beijing Oneder-Hightech Co. Ltd. was PEG2000 bearing a maleimide group at one end and two phosphate groups at the other end (Fig. S1b, Liu et al., 2013). The maleimide groups on the surface of UC NPs allowed further modification. The UC NPs were diluted using 10 mMpH 7.2 Tris–HCl buffer to a final concentration of 50 pM. TB-Apt C (100 μM) was added to the UC NPs solution to a final concentration of 0.5 nM. A $NaNO_3$ solution of 5 M in water was added to the former solution to produce a $NaNO_3$ concentration of 150 mM. The solution was left for 2 h to complete the modification of TB-Apt C to UC NPs. The modified UC NPs underwent ultrafiltration twice to remove excess DNA.

2.5. Assembly of the Au CORE-UC satellite sensor

To assemble the Au core-UC satellite nanostructure, purified TB-Apt-modified AuNPs were mixed with TB-Apt C-modified UC NPs at a 10:1 M excess relative to Au cores in hybridization buffer (10 mM pH 7.2 Tris–HCl). Particles were incubated at room temperature for 8 h.

2.6. Selectivity of the sensor

The selectivity of the as-fabricated sensor was evaluated by analyzing its selectivity for bovine serum albumin (BSA), human prostate-specific antigen (PSA), serum albumin (HSA), immunoglobulin G (IgG), and L-cysteine (L-cys). These proteins and amino acid were added to the sensor at a concentration of 1 ng/mL, and the operation procedures were the same as those for thrombin detection.

2.7. Recovery test of thrombin in Human serum

As serum is a complex biological matrix containing a wide variety of biomolecules, but no coagulation proteins such as thrombin, human serum was spiked with thrombin in the recovery test for further application. Human serum (obtained from Wuxi No. 2 People's Hospital, All the experiments were performed in compliance with the relevant laws and institutional guidelines, and the experiments were approved by the Ethics Committee of No. 2 People's Hospital) was diluted ten times with 10 mM Tris–HCl buffer (pH 7.2) as the analysis medium. Three concentrations of thrombin in the linear range (10, 20, 100 fg/mL) were spiked for monitoring.

3. Results and discussion

3.1. Establishment of fluorescence “off-on” switch for thrombin detection

As shown in Scheme 1, thrombin-aptamer (TB-Apt)-modified gold nanoparticles (AuNPs) were mixed with thrombin-aptamer complementary (TB-Apt C)-modified UC NPs, and the UC satellites assembly with Au cores led to core-satellites architecture, and the fluorescence of UC NPs was quenched. However, when thrombin was added to the system, the aptamer on the surface of AuNPs binding to the target then released the UCNPs thus the quenched fluorescence recovered, and a fluorescence “off-on” switch for thrombin determination was obtained.

During the assembly process, the fluorescence intensity was monitored and the corresponding assembly product was characterized by TEM. TEM images at different hybridization times (1, 2, 4 and 8 h) are shown in Fig. S2. The number of UC satellites around the Au core increased with prolonged hybridization time. Two layers of UC NPs can be observed around some Au NPs (Fig. S2b, d), which may be formed from the collapse of three dimensional structure of core-satellite during the drying in the sample preparation process for TEM characterization. The assembly of Au core-UC satellites took 8 h and a yield of up to 80% was achieved. The UC NPs showed three fluorescence peaks. The peak at 543 nm was chosen as the signal peak, because it was nearest the plasmonic band of AuNPs which exhibited excellent sensitivity.