Analytica Chimica Acta 689 (2011) 243–249

Contents lists available at ScienceDirect

Analytica Chimica Acta

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

Magnetic relaxation switch and colorimetric detection of thrombin using aptamer-functionalized gold-coated iron oxide nanoparticles

Guohai Liang[a], Shaoyu Cai[a], Peng Zhang[a], Youyuan Peng[b], Hui Chen[a], Song Zhang[a], Jilie Kong[a,*]

[a] Department of Chemistry and Institutes of Biomedical Sciences, Fudan University, Shanghai 200433, PR China
[b] Department of Chemistry, Quanzhou Normal University, Quanzhou 362000, PR China

ARTICLE INFO

Article history:
Received 5 November 2010
Received in revised form 16 January 2011
Accepted 20 January 2011
Available online 27 January 2011

Keywords:
Magnetic relaxation switch
Colorimetric sensor
Fe_3O_4@Au
Human α-thrombin

ABSTRACT

We describe a sensitive biosensing system combining magnetic relaxation switch diagnosis and colorimetric detection of human α-thrombin, based on the aptamer–protein interaction induced aggregation of Fe_3O_4@Au nanoparticles. To demonstrate the concept, gold-coated iron oxide nanoparticle was synthesized by iterative reduction of $HAuCl_4$ onto the dextran-coated Fe_3O_4 nanoparticles. The resulting core–shell structure had a flowerlike shape with pretty narrow size distribution (referred to as "nanorose"). The two aptamers corresponding to human α-thrombin were conjugated separately to two distinct nanorose populations. Once a solution containing human α-thrombin was introduced, the nanoroses switched from a well dispersed state to an aggregated one, leading to a change in the spin–spin relaxation time (T_2) as well as the UV–Vis absorption spectra of the solution. Thus the qualitative and quantitative detection method for human α-thrombin was established. The dual-mode detection is clearly advantageous in obtaining a more reliable result; the detection range is widened as well. By using the dual-mode detection method, a detectable T_2 change is observed with 1.0 nM human α-thrombin, and the detection range is from 1.6 nM to 30.4 nM.

1. Introduction

Due to the remarkable chemical and physical properties on the nanometer scale, various types of nanoparticles have been extensively studied for numerous biotechnological applications [1]. For example, magnetic nanoparticles are widely used in tissue imaging, drug delivery, molecular sensing and bio-separation. Since the first magnetic resonance based assay was developed by Weissleder and co-workers [2], superparamagnetic nanoparticle based biosensors, also known as magnetic relaxation switches (MRSw), have been designed to identify and quantify a wide variety of target analytes [3–9]. The potential to probe nonoptical samples may eliminate time-consuming and complex processing steps, which makes MRSw assay an attractive biosensing approach. The principle underlying the detection mechanism of MRSw assay is based on the switch of the magnetic nanoparticles between a dispersed and aggregated state upon target interaction, with a concomitant change in the spin–spin relaxation time (T_2) of the solution's water protons around the magnetic nanoparticles [10]. On the basis of changes in T_2 (δT_2) the analyte concentration can be determined. Thus the size distribution and the stability in solution are important factors in selecting superparamagnetic nanoparticles for use in MRSw assay application. Cross-linked iron oxide (CLIO) is the most usually used superparamagnetic nanoparticle, owing to its narrow size distribution and excellent stability in a variety of fluids. Now, a number of CLIO-based MRSw sensors have been developed [11]. For example, Westmeyer et al. [12] reported an enzyme reporter system using CLIO as a turn-on sensor. Osborne et al. [13] covalently attached spiropyran derivative to CLIO, which was used as a "smart" T_2 agent that was reversibly activated by a visible light stimulus. However, most of these sensors have linear T_2 response over a comparatively narrow concentration of analytes. Overtitration results in erratic T_2 values, which arise from the CLIO clusters becoming unstable in solution [5,10]. On the other hand, gold nanoparticle-based colorimetric biosensor has been increasingly applied for the detection of a large variety of targets. Sharing similar dispersion-to-aggregation process with MRSw assay, the gold nanoparticle-based colorimetric biosensor has a broader detection range, and typically the detection limit is in the range of nM to μM without signal amplification steps [14].

As we all know, the multifunctional gold-coated iron oxide nanoparticles (Fe_3O_4@Au) integrate excellent surface chemistry, special optical properties, and superparamagnetic properties in a core–shell structure, which makes them extremely interesting for optical, magnetic, and biomedical applications. So far, many research works on synthesis of Fe_3O_4@Au nanoparticles have been reported, and Fe_3O_4@Au nanoparticles with controlled plasmonic and magnetic properties can be successfully synthesized by various

* Corresponding author. Tel.: +86 21 6564 2405; fax: +86 21 6564 1740.
E-mail address: jlkong@fudan.edu.cn (J. Kong).

0003-2670/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.aca.2011.01.046

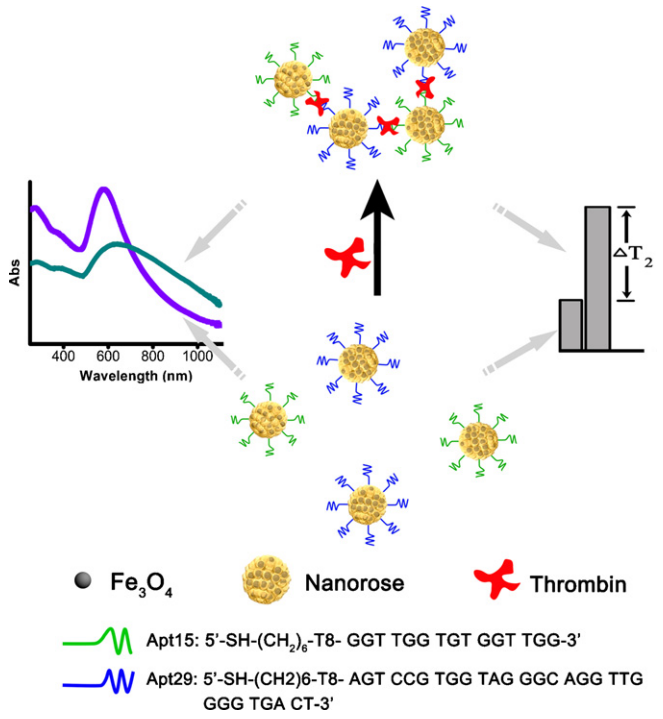

Fig. 1. Schematic diagram showing the principle of aptamer-linked sandwich assay for TB using Fe_3O_4@Au nanoparticles (nanorose).

methods [15–18]. We herein propose that the core–shell structures with combined magnetic and optical properties can provide a novel platform for MRSw assay, using the magnetic property, and colorimetric sensing, using the optical property. Thus the potential of such multifunctional nanoparticle is enhanced, and the application is broadened as well. The Fe_3O_4@Au nanoparticles can be excellent multifunctional sensors especially in aptamer-based protein detection. For one thing the Au shell provides enhanced stability and allows for convenient surface-modification with aptamer, for another we can get a δT_2 as well as a colorimetric change, by comparing the colorimetric result with the MRSw one, we are more confident to quantify the analytes. To the best of our knowledge there is no published paper using Fe_3O_4@Au nanoparticles for MRSw assay or colorimetric detection application.

Based on these principles, we herein report the application of Fe_3O_4@Au nanoparticles as double-functional sensors for the detection of protein. Flowerlike Fe_3O_4@Au nanoparticles reported by Ma et al. [15] were chosen as they could stay monodispersed in solution for a long time, also they were easy to synthesize. Human α-thrombin (TB) with its two binding aptamers was chosen as the target analyte of interest. TB is a common but critical protein that naturally functions as a blood clotting factor to convert fibrinogen to firin. Excessive TB will induce thrombosis while low content of TB will induce an excessive bleeding. Two aptamers have been identified to bind with TB: a 29-mer aptamer (Apt29), which binds to the heparin-binding site of TB ($K_d \approx 0.5$ nM), and a 15-mer aptamer (Apt15), which binds to the fibrinogen-binding site of TB ($K_d \approx 26$ nM) [19]. Fig. 1 schematically shows the procedure of the aptamer sandwich assay that is generally applicable to the analysis of proteins. To assay for TB, the thiolated aptamers were conjugated to nanoroses seperately through the strong Au–S bond. In the absence of TB, the aptamer-functionalized nanoroses (Apt29-nanoroses and Apt15-nanoroses) dispersed well in the solution. However, Once a solution containing TB was introduced, the nanoroses switched from a well dispersed state to an aggregated one, leading to a change in the spin–spin relaxation time (T_2) as well as the UV–Vis absorption spectra of the solution. Thus two distinct signals were obtained and a comparison between them could be made.

2. Experimental

2.1. Reagents

Chloroauri acid ($HAuCl_4{\cdot}4H_2O$), ferric chloride ($FeCl_3{\cdot}6H_2O$, 99.0%), ferrous chloride ($FeCl_2{\cdot}6H_2O$, 99.0%) and dextran ($M_W = 20{,}000\ g\ mol^{-1}$) were purchased from Shanghai Chem. Corp. Human α-thrombin and bovine serum albumin (BSA) were purchased from Aldrich. The aptamers of TB used in this study had the following sequences: Apt29, 5′-SH-$(CH_2)_6$-T8-AGT CCG TGG TAG GGC AGG TTG GGG TGA CT-3′; Apt15, 5′-SH-$(CH_2)_6$-T8-GGT TGG TGT GGT TGG-3′. They were synthesized and purified by Sangon Corp., Shanghai. DNA, TB and BSA were prepared in 10 mM Tris–HCl buffer (pH 7.4). Milli-Q water (18.2 MΩ-cm) was used throughout the experiments. All other chemicals were of analytical grade and used as received.

2.2. Instrumentation

T_2 was determined on a 0.47 T MRI instrument (Shanghai Niumag Corp.) at 38 °C. UV–Vis absorption spectra were recorded on an Agilent 8453 UV–Vis Spectrophotometer. Inductively coupled plasma-atomic emission spectroscopy (ICP-AES) was performed on a Thermo E. IRIS Duo ICP-OES spectroscopy. Transmission electron microscopy (TEM) and scanning electron microscopy (SEM) images were taken with a JEOL2011 microscope (Japan) operated at 200 kV and a Philips XL30 electron microscope (The Netherlands) operated at 10 kV, respectively. Energy-dispersive spectroscopy (EDS) spectrum was collected during TEM imaging.

2.3. Preparation of nanoroses

In order to prepare nanoroses, Fe_3O_4 nanoparticles were first synthesized by coprecipitation method as described [20]. In brief, 30 mL dextran aqueous solution (15%, w/w) was titrated with 8 mL NH_4OH (>25%, w/w) to pH11.7. The dextran solution was heated in a 150 mL flask with mechanical stirring to 25 °C in a water bath. Then 10 mL of freshly prepared 1.5 g of $FeCl_3{\cdot}6H_2O$ and 0.64 g of $FeCl_2{\cdot}4H_2O$ aqueous solution was injected into the dextran solution after passing through a hydrophilic 0.22 μm filter. The black suspension was stirred for 0.5 h. Then the large aggregates were removed by centrifugation at 10,000 rpm for 20 min. The supernatant was decanted and dialyzed against DI water for 24 h in a dialysis bag with 25 kDa molecular weight cut off. The concentration of iron oxide determined from ICP-AES was 0.60 mg Fe/mL.

Nanoroses were next grown by iterative reduction of $HAuCl_4$ onto iron oxide nanoparticles with hydroxylamine as a seeding agent. 5 mL of the as-prepared dextran-coated iron oxide solution was mixed with 4 mL DI water. 200 μL 1% hydroxylamine seeding agent and 0.5 g dextrose were added. The solution was magnetically stirred to 30 °C in a water bath for 15 min. Before starting the $HAuCl_4$ addition, 50 μL 7% NH_4OH solution was added to the iron oxide dispersion to tune the pH to 9.0. Four aliquots of 100 μL 0.25% $HAuCl_4$ aqueous solution were added to the stirred dispersion (with 10 min between additions). The color gradually changed from brown to dark brown during the four aliquot additions. After that the dense Fe_3O_4@Au particles were separated from the less dense uncoated particles by centrifugation at 8000 rpm for 6 min. The supernatant was decanted and the nanoroses were re-dispersed in dextran aqueous solution (5%, w/w) under sonication for 10 min. The centrifugation and re-dispersion steps were repeated for five times to remove the uncoated iron oxide nanoparticles, then two