Analytical Biochemistry 441 (2013) 95–100

Contents lists available at ScienceDirect

Analytical Biochemistry

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

A chronocoulometric aptasensor based on gold nanoparticles as a signal amplification strategy for detection of thrombin

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ARTICLE INFO

Article history:
Received 11 May 2013
Received in revised form 8 July 2013
Accepted 17 July 2013
Available online 26 July 2013

Keywords:
Aptasensor
Chronocoulometry
Gold nanoparticles
Thrombin

ABSTRACT

A sensitive chronocoulometric aptasensor for the detection of thrombin has been developed based on gold nanoparticle amplification. The functional gold nanoparticles, loaded with link DNA (LDNA) and report DNA (RDNA), were immobilized on an electrode by thrombin aptamers performing as a recognition element and capture probe. LDNA was complementary to the thrombin aptamers and RDNA was noncomplementary, but could combine with $[Ru(NH_3)_6]^{3+}$ (RuHex) cations. Electrochemical signals obtained by RuHex that bound quantitatively to the negatively charged phosphate backbone of DNA via electrostatic interactions were measured by chronocoulometry. In the presence of thrombin, the combination of thrombin and thrombin aptamers and the release of the functional gold nanoparticles could induce a significant decrease in chronocoulometric signal. The incorporation of gold nanoparticles in the chronocoulometric aptasensor significantly enhanced the sensitivity. The performance of the aptasensor was further increased by the optimization of the surface density of aptamers. Under optimum conditions, the chronocoulometric aptasensor exhibited a wide linear response range of 0.1–18.5 nM with a detection limit of 30 pM. The results demonstrated that this nanoparticle-based amplification strategy offers a simple and effective approach to detect thrombin.

The sensitive and quantitative analysis of proteins is very important in disease diagnosis and biomedical research. The establishment of a reliable detection method for proteins has attracted the efforts of the scientific community [1,2]. In many immunoassay techniques, antibodies are frequently used for the recognition of proteins because of their high specificity. In recent years, aptamers, a new class of single-stranded DNA (ssDNA) or RNA oligonucleotides obtained through a method called "systematic evolution of ligands by exponential enrichment" from random DNA or RNA libraries [3,4], have become an alternative in analytical bioassays as protein recognition elements. Compared with antibodies, aptamers present some advantages, such as relatively easy production, excellent target versatility, specific binding, high stability in complex physical and chemical environments, long-term storage, reversible thermodynamic denaturation, and convenient regeneration [5–8].

Thrombin, which is a specific serine protease involved in the coagulation cascade, thrombosis, and hemostasis, has attracted much interest because of its superior functions such as converting soluble fibrinogen into insoluble strands of fibrin and catalyzing many other coagulation-related reactions [9,10]. In addition to its key role in the dynamic process of thrombus formation, thrombin has a pronounced proinflammatory character, which may influence the beginning and progression of atherosclerosis [11]. Therefore, a sensitive and specific method for the detection of thrombin at low concentration is necessary. So far, various strategies and technologies using aptasensors for thrombin have been developed, such as fluorescence [12,13], electrochemiluminescence [14], quartz crystal microbalance [15], surface plasmon resonance [16], and electrochemistry [17–21]. Among these, the electrochemical method has attracted much attention in the development of aptasensors because of its simple instrumentation, high sensitivity, fast response, and low cost.

To realize ultrasensitive detection, signal amplification of the electrochemical aptasensor is essential. Many methods for signal amplification have been introduced, such as rolling circle amplification [22–24], enzyme-based amplification [25–27], strand displacement amplification [28], and so on. However, because of the complicated protocols, high cost, and rigorous detection conditions, these methods for signal amplification are disadvantageous. Recently, metal nanoparticles used in signal amplification [29,30], especially gold nanoparticles (AuNP's), have attracted great attention because of their facile synthesis, large specific surface area, high chemical stability, favorable biocompatibility, good conductivity, and high affinity of binding to amine/thiol-containing molecules [31].

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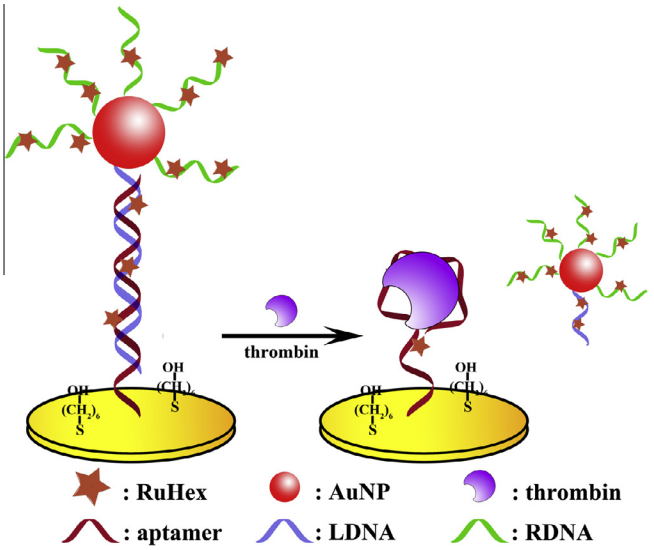
http://dx.doi.org/10.1016/j.ab.2013.07.023



Scheme 1. Schematic diagram for the fabrication of a chronocoulometric aptasensor based on gold nanoparticle amplification for the detection of thrombin.

Herein, we report a novel strategy to develop a sensitive chronocoulometric aptasensor based on gold nanoparticle amplification for the detection of thrombin. The principle of the chronocoulometric aptasensor for the detection of thrombin is illustrated in Scheme 1. First, thrombin aptamers were self-assembled on the surface of a gold electrode to form a monolayer. The DNA-functionalized AuNP's were immobilized on a thrombin aptamer-modified gold electrode by DNA hybridization. Different from the reported AuNP's with one kind of DNA, AuNP's labeled with two kinds of DNA were used here. Since link DNA (LDNA) was complementary to aptamers and report DNA (RDNA) was non-complementary, the low density of LDNA on AuNP's would be favorable to the one-to-one combination of aptamers. In the absence of thrombin, the functional gold nanoparticles (DNA–AuNP's) localized at the electrode surface could electrostatically adsorb abundant hexaaminеruthenium(III) chloride ($[Ru(NH_3)_6]^{3+}$; RuHex) cations, which serve as the signaling molecules. After thrombin was added, as aptamers could bind tightly to target molecules to form a tertiary complex with a binding constant greater than that of the DNA duplex, the DNA–AuNP's were replaced by the target to form the target–aptamer complex. The release of DNA–AuNP's was accompanied by the extrication of the abundant RuHex molecules, which induced a significant decrease in chronocoulometric signal. By employing this strategy, we demonstrated that this chronocoulometric aptasensor can sensitively and selectively detect thrombin.

Experimental procedures

Reagents

All oligonucleotides were synthesized and purified by Sangon, Inc. (Shanghai, China). The sequences of the single-stranded oligonucleotides were as follows: link DNA, 5′-SH-$(CH_2)_6$-AACCAACCACAC-3′; report DNA, 5′-SH-$(CH_2)_6$-TTTTTTTCGGCCTGTTCCGG-3′; aptamer, 3′-TTGGTTGGTGTGGTTGGTTT-$(CH_2)_6$-SH-5′.

Mercaptohexanol (MCH), RuHex, thrombin, and tris(2-carboxyethyl)phosphine hydrochloride (TCEP) were purchased from Sigma (St. Louis, MO, USA). Tris(hydroxymethyl)aminomethane (Tris) and $AuCl_3HCl{\cdot}4H_2O$ were obtained from Aladdin Chemistry Co., Ltd. (Shanghai, China). Lysozyme and bovine serum albumin were purchased from Beijing Dingguo Changsheng Biotechnology Co., Ltd. (China). All reagents were of analytical grade and used without further purification. All solutions were prepared with doubly distilled water (18.2 MΩ cm). All glassware used in the following procedure was cleaned in a bath of freshly prepared 3:1 HCl:HNO_3 and then rinsed thoroughly with doubly distilled water.

Apparatus

Cyclic voltammetry (CV), electrochemical impedance spectroscopy, and chronocoulometry (CC) were performed with a CHI 660B electrochemical workstation (Shanghai Chenhua Instrument Corp., China). Differential pulse voltammetry was performed with an Autolab PGSTAT302 electrochemical workstation (Eco Chemie BV, Utrecht, The Netherlands). A conventional three-electrode cell was employed, which involved a gold working electrode (2 mm in diameter), a platinum foil counterelectrode, and a saturated calomel reference electrode (SCE). All the potentials in this paper are given with respect to SCE. The electrolyte buffer was thoroughly purged with nitrogen before experiments. Transmission electron microscopy (TEM) images were taken using a Hitachi 600 (Hitachi Ltd., Tokyo, Japan).

Preparation of gold nanoparticles

Gold nanoparticles were prepared by citrate reduction of $HAuCl_4$ according to the literature [32]. Briefly, 10 ml of 38.8 mM sodium citrate was immediately added to 100 ml of 1.0 mM $HAuCl_4$ refluxing solution under stirring, and the mixture was kept boiling for another 15 min. The solution color turned to wine red and was cooled to room temperature with continuous stirring. The size of the AuNP's was verified by transmission electron micrograph and the diameter was 12 ± 2.0 nm. The TEM image of AuNP's is shown in Fig. 1.

Functionalization of gold nanoparticles with DNA

The immobilization of LDNA and RDNA on AuNP's was carried out as follows. Briefly, a mixture of LDNA (5 μM) and RDNA (20 μM) was activated with 4 μl of 10 mM acetate buffer (pH 5.2)

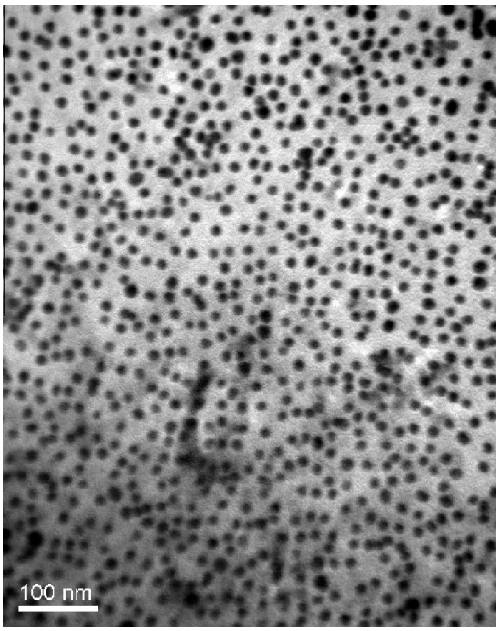


Fig.1. TEM image of AuNP's.