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Short communication

Electrochemical detection of DNA hybridization based on signal DNA probe modified with Au and apoferritin nanoparticles

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ABSTRACT

A novel and ultrasensitive electrochemical approach for sequence-specific DNA detection based on signal dual-amplification with Au NPs and marker-loaded apoferritin NPs was reported. Target DNA was sandwiched between capture DNA coupled to magnetic beads and signal DNA self-assembled on Au NPs which were incorporated with marker-loaded apoferritin NPs. Subsequent electrochemical stripping analysis of the electroactive markers released from apoferritin NPs in acidic buffers provided a means to quantify the concentration of target DNA. In this means, one target signal could be transformed into multiple redox signals of the markers since a single Au NP could be loaded with dozens of apoferritin NPs, and an apoferritin NP could be loaded with thousands of markers. Under the optimum conditions, the linear range was from 2.0×10^{-16} to 1.0×10^{-14} M and the detection limit was 5.1×10^{-17} M by using the cadmium as a model marker. The proposed DNA biosensor not only exhibited excellent sensitivity but also had good reproducibility and selectivity against two-base mismatched DNA.

1. Introduction

It is well known that sequence-specific DNA detection is essential for clinical diagnosis, pathology, and genetics (Butler and Forensic, 2006; Leslie et al., 2007; Shinsuke et al., 2005; Stratton et al., 2004). In recent years, great efforts have been made to improve sensitivity of DNA detection. With the rapid development of nanotechnologies, metal or semiconductor nanoparticles (NPs) (Hansen et al., 2006; Liu and Lin, 2005; Z. Zhu et al., 2008; He et al., 2004) with unique optical and electrical properties have been widely used as labels or indicators for the amplified detection of DNA. Hu et al. (2008) have developed an electrochemical DNA biosensor based on nanoporous gold electrode and multifunctional encoded DNA–Au bio bar codes, which can detect as low as 28 aM DNA. Ding's group have reported an ultrasensitive chemiluminescence method for the detection of single-nucleotide polymorphisms (SNPs) by using monobase-modified Au and CuS nanoparticles as labels, which can detect as low as 19 aM SNPs (Ding et al., 2010). Due to the already existing achievements in the development of ultrasensitive hybridization assays, parameters such as simplicity, rapidity, reliability, multiplexing, high-throughput, etc. are of great importance. Among of many detection techniques such as fluorescence (Gerion et al., 2003), chemiluminescence (CL) (Ding et al., 2008), electrogenerated chemiluminescence (ECL) (D.B. Zhu et al., 2008), surface plasmon resonance (Lee et al., 2007), and quartz crystal microbalance (Yao et al., 2008), electrochemical detection plays more important parts in the analysis of biological systems for its good sensitivity, rapidity, easy process, and low cost (Chang et al., 2007; Elsholz et al., 2009; Hu et al., 2008; Kara et al., 2004; Wong and Gooding, 2006).

Apoferritin is only composed of a protein shell of ferrition, so it has a hollow cagelike structure. The inner and outer diameters of the protein shell are about 8 and 12 nm, respectively. Apoferritin is stable in neutral or alkaline environment, whereas it can be dissociated into 24 polypeptide subunits at acidic environment. On the other hand, these polypeptide subunits can reconstitute apoferritin when pH is raised to 8.5 (Aime et al., 2002; Dominguez-Vera and Colacio, 2003). In view of its unique cavity structure as well as its dissociation and reconstitution characteristics at different pH, apoferritin has been widely used as a protein cage to synthesize size-restricted and uniform NPs, e.g., cobalt (Allen et al., 2003), cadmium sulfide (Wong and Mann, 1996), and zinc selenide (Iwahori et al., 2005). Furthermore, fluorescence or redox markers can be captured in the cavity of apoferritin, and marker-loaded apoferritin NPs as labels have been developed for highly sensitive protein assay (Liu et al., 2006a,b). Recently, Lin's group have used apoferritin NPs encapsulating metal phosphate as labels for electrochemical quantification of SNPs (Liu and Lin, 2007).

In the present study, we report a new amplification strategy for the determination of sequence-specific DNA based on signal DNA probe modified with Au NPs and marker-loaded apoferritin NPs. Taking advantages of signal dual-amplification of Au and apo-

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ferritin NPs, one target signal can be transformed into multiple redox signals of the markers. The experimental results have demonstrated that the new amplification strategy can detect as low as femtomolar target DNA and has exhibited good selectivity against two-base mismatched DNA. The proposed biosensor is compared with other reported and sensitive sensors for the detection of DNA hybridization listed by Li et al. (2009) and Zhang et al. (2008). Moreover, the novel amplification technique with the characteristic of using the nanostructured protein as labels has excellent biocompatibility as well as convenient, rapid detection.

2. Experimental

2.1. Materials and chemicals

All of synthetic oligonucleotides were purchased from SBS Genetech Co., Ltd. (Beijing, China) with the following sequences: capture DNA (S_1), 3′-NH_2-CTG ATC TGA GTA GAC ACT-5′; target DNA (S_2), 5′-ACT CAT CTG TGA GTT TTC GCT CGT GTG AGC-3′; target binding DNA (signal DNA, S_3), 3′-CGA GCA CAC TCG AAT CTG-SH-5′; two-base mismatched DNA (S_4), 5′-ACT CAT GAG TGA GTT TTC GCT CGT GTG AGC-3′; non-complementary DNA (non-cDNA, S_5), 5′-CAG TGC ACT GAC TAC GCA TAC ATA TGA CAT-3′.

Carboxyl-modified magnetic beads (MBs, diameter 1.0 μm, 20 mg mL^{-1}) were ordered from Tianjin Baseline Chromtech Research Centre (China). Apoferritin, tri-(2-carboxyethl) phosphine hydrochloride (TCEP), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC), and Tris–HCl buffer were purchased from Sigma (USA). $HAuCl_4$ was obtained from Tianjin Yingda Sparseness & Noble Reagent Chemical Factory (China). All reagents were of analytical grade and used without further purification. Doubly distilled and deionized water was used throughout this work.

2.2. Apparatus

The electrochemical measurements were performed on a CHI 832B electrochemical analyzer (Shanghai CH Instrument Company, China) with a three-electrode system that consisted of a platinum wire as auxiliary electrode, an Ag/AgCl electrode as reference electrode, and an in situ plated mercury film glassy carbon electrode as working electrode. Transmission electron microscopy (TEM) images were taken with a JEOL JSM-6700F instrument (Hitachi). The disposable PD-10 desalting column packed with Sephadex G-25 medium (exclusion limit 5000) was bought from Tianjin Baseline Chromtech Research Centre (China) and used to purify the protein solution. Centrifugal filter devices (Amicon Ultra-15, 30,000 MWCO, Millipore Corp.) were used to separate and concentrate the apoferritin solution.

2.3. Preparation of cadmium phosphate-loaded apoferritin (CPLA) NPs, Au NPs, and DNA–Au-CPLA conjugates

CPLA NPs were prepared according to the literature (Liu et al., 2006b). Au NPs were prepared by citrate reduction of $HAuCl_4$ according to the method reported previously (Liu and Lu, 2006). DNA–Au-CPLA conjugates were prepared by following the published procedure with a slight modification (Taton et al., 2000) (Supplementary data).

2.4. Fabrication of the biosensor

The oligomer-coated MBs were prepared according to the literature (Miao et al., 2008). 50 μL suspension of carboxyl-modified MBs were activated in 100 μL of 0.1 M imidazole buffer (pH 6.0) containing 0.2 M EDC with gentle shaking for 60 min. Subsequently, 200 μL of 1×10^{-6} M capture DNA (S_1) was added and incubated for 1 h at 37 °C with gentle mixing. The resulting probes were washed three times with 100 μL PBS buffer (pH 7.4) and resuspended in 50 μL PBS buffer. Corresponding amounts of target DNA (S_2) was added for the hybridization reaction. After hybridization for 1 h at 37 °C, the resulting MBs were washed as above. Then 100 μL DNA–Au-CPLA conjugates solution was added for the second hybridization between target DNA (S_2) and signal DNA (S_3) for 1 h at 37 °C. After hybridization, the resulting MBs were separated magnetically from the solution and washed twice with PBS buffer.

2.5. Electrochemical detection

The above MBs were dispersed in 400 μL of 0.1 M acetate buffer (pH 4.6) containing 10 μg mL^{-1} $HgCl_2$ with gentle shaking. After mixing for 5 min and a subsequent magnetic separation, stripping voltammetric measurements of the dissolved Cd^{2+} were performed using an in situ plated mercury film on a glassy carbon electrode following a pretreatment at 0.6 V for 1 min, and an accumulation at −1.4 V for 5 min. After a 15 s rest period, the differential pulse voltammetry (DPV) was performed by scanning the potential from −0.8 to −0.5 V (vs. Ag/AgCl) with a pulse amplitude of 50 mV and a pulse width of 50 ms. The anodic stripping peak current located at −0.66 V was taken as the analytical response.

3. Results and discussion

3.1. Fabrication of the biosensor and detection process

The principle of the protocol presented in this work was shown in Fig. 1. A sandwich-type DNA hybridization strategy was employed. NH_2-functionalized capture DNA (S_1) was first immobilized on carboxyl-modified MBs, and hybridized with target DNA (S_2), which further hybridized with signal DNA (S_3) modified with Au NPs and marker-loaded apoferritin NPs. After magnetic separation from unwanted DNA–Au-CPLA conjugates, the markers were released from apoferritin NPs into acidic buffer. Subsequent electrochemical stripping analysis of the electroactive markers provided a means to quantify the concentration of target DNA.

In this means, one target signal can be transformed into multiple redox signals of the markers since a single Au NP can be loaded with dozens of apoferritin NPs, and an apoferritin NP can be loaded with about 1350 cadmium ions (Liu et al., 2007). In acetate buffer, apoferritin NPs can be disassociated into subunits and release cadmium phosphate which is subsequently dissociated into dihydrogenphosphate anions and cadmium ions, and this process can be determined by stripping voltammetry. Taking advantages of dual-amplification of Au and apoferritin NPs, the new design for detection of DNA hybridization significantly enhances the sensitivity. A preconcentration process of cadmium ions performed by anodic stripping voltammetry (ASV) technology further increases the sensor performance.

3.2. Characterization of CPLA NPs, Au NPs and the biosensor

TEM was used to confirm that cadmium phosphate was loaded on apoferritin. The TEM images of apoferritin NPs and the synthesized CPLA NPs were shown in Supplementary data, Fig. 1A and Supplementary data, Fig. 1B, respectively. The apoferritin NPs with an uniform size of ~12 nm in diameter were transparent. Whereas, the synthesized CPLA NPs had cores which appeared black and were surrounded by the apoferritin protein shells. This showed cadmium phosphate was encapsulated in the apoferritin as expectation. The prepared Au NPs had an average diameter of approximate 20 nm as measured by TEM as shown in Supplementary data, Fig. 1C. The TEM of a MB fabricated with Au NPs probes was shown in Supplementary