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Short communication

Electrochemical detection of DNA hybridization based on bio-bar code method

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ABSTRACT

Based on the electrochemical detection of cadmium ions dissolved from CdS nanoparticles (NPs), a novel and sensitive assay for the sequence-specific DNA detection has been developed by using bio-bar code techniques. The "sandwich-type" DNA complexes were fabricated with the thiol-functionalized capture DNA firstly immobilized on the modified electrode (nanoAu-GCE) and hybridized with one end of target DNA, the other end of which was recognized with signal DNA labeled on the surface of Au NP. To amplify the detection signals, the Au NP was also modified with CdS NPs by an amidization reaction between bio-bar code binding DNA on the surface of Au NPs and mercaptoacetic acid on the surface of CdS NPs. The hybridization events were monitored by the electrochemical detection after the cadmium ions were dissolved from the hybrids. Under optimum conditions, the peak current value was increased with the increase of the concentration of target DNA in the range of 1.0×10^{-14} to 1.0×10^{-13} M. A detection limit of 4.2×10^{-15} M of target DNA was achieved. The ASV currents of two-base mismatched sequences and non-complementary sequences were also detected. The experiments indicated that two-base mismatched sequences showed weaker peak current and non-complementary sequences gave no response at all.

1. Introduction

Sequence-specific detection of DNA targets has become increasingly important for the diagnosis and treatment of genetic diseases, and forensic analysis (Endo et al., 2005; Drummond et al., 2003; Dubus et al., 2006; Bowden et al., 2005; Miao and Bard, 2004; Brettell et al., 2003). Many optical (Cao et al., 2002; Li and Rothberg, 2004; Pavlov et al., 2005; Ho et al., 2005; Peng et al., 2007), chemiluminescence (Miao and Bard, 2004; Zhang et al., 2008a,b; Ding et al., 2008), surface plasmon resonance (Fang et al., 2006; Lee et al., 2007), quartz crystal microbalance (Yao et al., 2008; Wu et al., 2007; Lu and Jiang, 2007), and electrochemical (Zhang et al., 2008a,b; Hu et al., doi:10.1021/ac8017197; Boon et al., 2000; Rijiravanich et al., 2008) techniques have been used for detecting and quantifying sequence-specific DNA. In these DNA detection processes, the amplification steps are important to realize the ultimate in terms of sensitivity. These amplification systems include polymerase chain reaction (PCR) protocols, and signal-amplification systems, such as fluorogenic substrate-active enzymes (Patolsky et al., 2002), modified liposomes (Cao et al., 2002), and nanoparticles (Brakmann, 2004).

The bio-bar code amplification assay created by Mirkin et al. is the only biodetection method that has the PCR-like sensitivity for both protein and nucleic acid targets without a need for enzymatic amplification (Rosi and Mirkin, 2005; Nam et al., 2002, 2003, 2004; Thaxton et al., 2005; Hill et al., 2007). The typical bio-bar code assay has been described in many reports. The assay has exhibited low-attomolar sensitivity for a variety of protein targets and high-zeptomolar sensitivity for nucleic acid targets when paired with scanometric readout.

However, current bio-bar code detection schemes still require microarrayer-based immobilization of oligonucleotide on a glass chip, surface passivation chemistry to minimize non-specific binding, silver enhancement of immobilized gold nanoparticles on a chip, light-scattering measurement, and a quantification step. Importantly, sophisticated instruments, such as microarrayers and chip-imaging tools limit portability, and the assay cost is bound to be expensive. To overcome the weak points, some novel bio-bar code detection methods have been reported recently (He et al., 2007; Zhu et al., 2008; Chang et al., 2006; Bao et al., 2006; Nam et al., 2005).

Within recent years, several inventive designs for DNA sensors based on an electrochemical readout have appeared due to the fact that electrochemical detectors are simple, reliable, cheap, sensitive, and selective for genetic detection (Katz and Willner, 2004). Electrochemical sensors based on impedance (Pan and Rothberg, 2005) or voltammetry (Anne et al., 2003) have been reported. Wang et al. (2001) have demonstrated for the first time the use of

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electrochemical stripping metal analysis for monitoring DNA hybridization. The target DNA used in this method was modified with biotin to conjugate the streptavidin gold. In another of their work (Wang et al., 2003), a multi-target electrochemical DNA detection based on the use of different inorganic-colloid (quantum dots) tags has been constructed, but the detect limitation was not low enough.

Our group has constructed an electrochemical bio-bar code assay based on DNA-modified gold nanoparticles (Au NPs) provides a non-enzymatic method for quantitative detection of protein. Using AFP detection as a model, the detection limit of this assay for AFP determined is 9.6 pg/mL, 100-fold improvement compared to the best enzyme-linked immunosorbent assay (ELISA) system.

Herein, we report an electrochemical bio-bar code assay for the quantitative detection of DNA hybridization. The present work consists of GCE-nanoAu electrode modified with capture DNA, Au-NPs modified with signal DNA and CdS NPs labeled bio-bar code DNA. The capture DNA and the signal DNA are complementary to the two end of the target DNA, respectively, to conform a sandwich DNA bioassay. The detection of target DNA is realized by indirect quantification of the cadmium ions dissolved from the CdS NPs on the hybrids by anodic stripping voltammetry (ASV). This method can quantitatively detect DNA with high speed and sensitivity, and the detection limit of current work is as low as 4.2×10^{-15} M.

2. Experimental

2.1. Reagents

The synthetic oligonucleotides were purchased from SBS Genetech. Co. Ltd., China. The two-base mismatched DNA sequences, 5′-ACT GCT AGA GAT TTT CCA CAC TGA CTA AAA GCG TCT GTG GGA-3′, the non-complementary DNA sequences, 5′-ACT GCT AGA GAT TTT CCA CAC TGA CTA CTT CAA CAG TGC CCC-3′.

1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC), hydrogentetrachloroaurate(III) trihydrate ($HAuCl_4 \cdot 3H_2O$), trisodium citrate and Tris-(2-carboxyethyl)phosphine hydrochloride (TCEP) were purchased from Sigma–Aldrich. Imidazole was obtained from Guoyao Chemical Co. Mercaptoacetic acid was obtained from Yuanhang Chemical Co. 6-Mercapto-1-hexanol (MCH) was obtained from Fluka. All the reagents were analytical grade and used without further purification. The 0.1 M PBS buffer (pH 7.4), 0.1 M Tris–HCl buffer (pH 6.8), and 0.1 M phosphate buffer containing 1‰ SDS (pH 7.0) were prepared by standard methods. Deionized and doubly distilled water was used throughout.

2.2. Apparatus

The electrochemical measurements for cyclic voltammetry (CV), electrochemical impedance spectroscopy (EIS) and stripping voltammetry were carried out on a CHI 660C electrochemical working station (CH Instrument Company, USA) using a three-electrode system consisted of a platinum wire as an auxiliary electrode, an Ag/AgCl electrode as reference electrode, a 4-mm-diameter Au disk electrode as working electrode (for CV and EIS), and a glassy carbon electrode as working electrode (for stripping voltammetry). UV–visible spectra were carried out on a Cary 50 UV–vis-NIR spectrophotometer (Varian). Transmission electron microscopy (TEM) image was taken with a JEOL JSM-6700F instrument (Hitachi).

2.3. Preparation of Au NPs and water-soluble CdS NPs

Au NPs and mercaptoacetic acid-capped CdS NPs were prepared according to the methods reported previously with a slight modification (Frens, 1973; Jie et al., 2007). Details were described in Supplementary Material.

2.4. Preparation of bio-bar code DNA probe modified with Au NPs and CdS NPs

The oligonucleotide-modified Au NPs were prepared according to the reference Nam et al. (2004). Briefly, 500 μL 1.025×10^{-6} M of 5′-thiol-functionalized DNA (the ratio of signal DNA to bio-bar code DNA is 1:70) were added to 3 mL of the prepared gold colloid solution. The 5′-thiol-functionalized DNA should be activated with 10 mM TCEP before use (Liu et al., 2007). After shaking gently for 16 h, the solution was allowed to stand for another 40 h, followed by centrifugation for at least 30 min at 10,000 rpm to remove excess reagents. Following removal of the supernatant, the red oily precipitate was washed with 5 mL of 0.1 M pH 7.0 phosphate buffer containing 0.1 M NaCl, recentrifuged, and redispersed in 3 mL of 0.1 M pH 7.0 phosphate buffer containing 0.3 M NaCl. The solution of prepared Au NP functionalized oligonucleotide probes was stored at 4 °C.

80 μL of a 0.1 M imidazole solution (pH 6.8) was added to the Au NPs functionalized oligonucleotide probes solution for 30 min, then 40 μL of 0.1 M EDC and 500 μL of CdS colloid (pH 4.0) were added. The labeling reaction was incubated at room temperature for 12 h with stirring. Finally the nanoparticles were isolated by centrifugation at 10,000 rpm for 15 min at 4 °C. The precipitate was washed with water, recentrifuged and redispersed in water. The bio-bar code DNA probe solution was stored at 4 °C.

2.5. Fabrication of the biosensor

A glassy carbon electrode was polished carefully with alumina slurries (1, 0.3, 0.05 μm) and washed ultrasonically with deionized and doubly distilled water. The glassy carbon electrode was immersed into 6 mM $HAuCl_4$ solution containing 0.1 M KNO_3 (prepared in doubly distilled water, and deaerated by bubbled with nitrogen). A constant potential of −0.4 V versus Ag/AgCl was applied for 400 s. Then, the modified electrode (nanoAu-GCE) was washed with doubly distilled water and dried carefully. Then it was electrochemically cleaned in 0.5 M H_2SO_4 solution by cyclic potential scanning between 0.3 and 1.5 V until a standard CV was obtained. Subsequently, the electrode was rinsed with deionized and doubly distilled water and absolute ethanol in turn. The DNA self-assembly process was performed under potential control because it was shown that the application of low positive potentials to the gold surface accelerates the chemisorption process and may assist in organizing the monolayer (Wang and Liu, 2007). Subsequently, the electrode was immersed in 1 mL of 0.1 M Tris–HCl buffer containing 0.025 mM capture DNA and incubated for 5 min under an applied potential of 0.4 V. In order to avoid consequent non-specific adsorption in the following hybridization steps, the modified electrode was immersed in 0.1 M PBS buffer (pH 7.4) containing 0.2 mM MCH for 1 h to block the uncovered gold surface. The sandwich-type format assay used consists of two steps. First, the modified electrode was immersed into 0.1 M PBS buffer containing target DNA with the different concentrations at 37 °C. One hour later, the modified electrode was taken out and immersed into 0.1 M PBS buffer containing bio-bar code DNA probe modified with Au NPs and CdS NPs for 12 h. Rinsing the electrode surface with 0.1 M phosphate buffer containing 1‰ SDS (pH 7.0) after each step of the fabrication process is very important to remove non-specifically adsorbed sequences.

2.6. Electrochemical detection

The Au electrode modified with sandwich-type hybrids labeled with Au and CdS NPs was immersed into a colorimetric tube