Biosensors and Bioelectronics 26 (2011) 4601–4604

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

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

Short communication

Ferrocenemonocarboxylic–HRP@Pt nanoparticles labeled RCA for multiple amplification of electro-immunosensing

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ARTICLE INFO

Article history:
Received 25 February 2011
Received in revised form 24 April 2011
Accepted 25 April 2011
Available online 6 May 2011

Keywords:
Electrochemical immunoassay
Rolling circle amplification
HRP
Au nanoparticle
Pt nanoparticle

ABSTRACT

A multiple amplification immunoassay was proposed to detect alpha-fetoprotein (AFP), which was based on ferrocenemonocarboxylic–HRP conjugated on Pt nanoparticles as labels for rolling circle amplification (RCA). Firstly, the capture antibody (anti-AFP) was immobilized on glass carbon electrode (GCE) deposited nano-sized gold particles. After a typical immuno-sandwich protocol, primary DNA was immobilized by labeling secondary antibody, which acted as a precursor to initiate RCA. The products of RCA provide large amount of sites to link detection DNAs, which were labeled by signal probes (ferrocenemonocarboxylic) and horseradish peroxidase (HRP). Moreover, the enzymatic amplification signals could be produced by the catalysis of HRP and Pt nanoparticles with the addition of H_2O_2. These lead to multiple amplification signals monitoring by electrochemical instrument and further resulted in high sensitivity of the immunoassay with the detection limit of 1.7 pg/mL.

1. Introduction

Alpha-fetoprotein (AFP) is reported to be a tumor-associated fetal protein in the mid-1960s, which is synthesized by embryonic liver and yolk sac but absent or present only in low concentration in serum of healthy adult individuals. The increase of AFP concentration was demonstrated to be clinical utility for the diagnose of cancer and a birth defect screening agent (Carlini et al., 2007; Stefanova et al., 1988; Yakimenko et al., 2001). Although various approaches have been proposed for AFP detection (Matsuya et al., 2003; Stephan et al., 2006), the detection of tumor makers at the lowest level is still a great challenge. Owing to its efficiency, low-cost and sensitivity, electrochemical immunoassays have aroused much attention, and great efforts have been made to improve the detecting sensitivity by employing enzymes, nanoparticles, supramolecular polymerization and nanocontainers as signal amplifiers (Zhu et al., 2009; Wang et al., 2008; Caballero et al., 2009). Amongst them, DNA based amplification technology has been interesting for the reason that DNA could be manipulated by DNA enzymes and predictability of molecular interactions (Ou et al., 2009; Lee et al., 2010; Cheng et al., 2010; Zhao et al., 2006). Proverbially, polymerase chain reaction (PCR) is proved to be a common amplification technique for detection of various target molecules (Schweitzer et al., 2002; Ostuni et al., 2001; Feng et al., 2010). However, the disadvantages limit the application of the technique for the diagnostic, such as complicated and time consuming procedures. Compared to PCR, rolling circle amplification (RCA) exhibits some attractive advantages besides high selectivity and sensitivity. For instance, RCA is an isothermal enzymatic process, which prohibits the requirement of special laboratory conditions for the thermal circling. Additionally, by RCA, a padlock template circle hybridizes to a small nucleic acid which in turn prolonged around the circle, ultimately resulting in a long single stranded DNA (ssDNA) with repetitious sequence (Zhao et al., 2008; Li et al., 2010). The repetitious units on ssDNA could be detected by another DNA labeled by signal materials through the base paring reaction. Based on these merits, RCA has performed as an exquisite and simple molecular biology approach for protein detection on account of stringent requirement of high amplification efficiency (Wang et al., 2008; Caballero et al., 2009; Ou et al., 2009).

In this communication, we prove a sensitive electrochemical immunoassay based on multiple amplification strategy containing RCA technology, nanomaterial based amplification and enzyme associated signal enhancement. After a typical immuno-sandwich protocol, primary DNA was immobilized by labeling secondary antibody (Ab_2), which acted as a precursor to initiate RCA. The products of RCA provide large amount of sites to link detection DNAs, which were labeled by signal probes (ferrocenemonocarboxylic) and HRP. Moreover, the enzymatic amplification signals could be produced by the catalysis of HRP and Pt nanoparticles with the addition of H_2O_2. These lead to multiple amplified signals and low detection limit of the AFP immunosensor.

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doi:10.1016/j.bios.2011.04.043

2. Experimental

2.1. Material and apparatus

Monoclonal AFP antibody pairs were purchased from Biocell Company (Zhengzhou, China), stored in the frozen state. Bovine serum albumin (BSA) (96–99%), HRP, gold chloride, chloroplatinic acid, sodium citrate, Tween 20, were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Primary DNA (pDNA; sequence: 5′-SH-TTT TTT TTT TTT TGT GTC TCC TAT CCT GTA), padlock template (sequence: 5′-p-GA GTC GAC ACA TTG TTG TAC TTC TAA CAT CCA GTC TTG AGT GGA CAA TCT TTG ACA CTT CTA GCG TAT AAT ACA GGA TAG) and detection DNA (dDNA; sequence: 5′-SH-AG TCT TGA GTG GAC AAT C) with chemical decoration were synthesized by Takara Biotechnology Co. Ltd. (Dalian, China). Phi29 DNA polymerase, T4 DNA ligase, deoxyribonucleoside 5′-triphosphates mixture (dNTP) were purchased from Fermentas (Lithuania). CHI 660D electrochemistry workstation (Shanghai CH Instruments, China) was used to carry out the electrochemical measurements. The three-compartment electrochemical cell was applied, which contained a platinum wire auxiliary electrode, a saturated calomel reference electrode (SCEs) and the working electrode (glass carbon electrode) (4 mm).

2.2. The synthesis of pDNA@Au labeled Ab_2

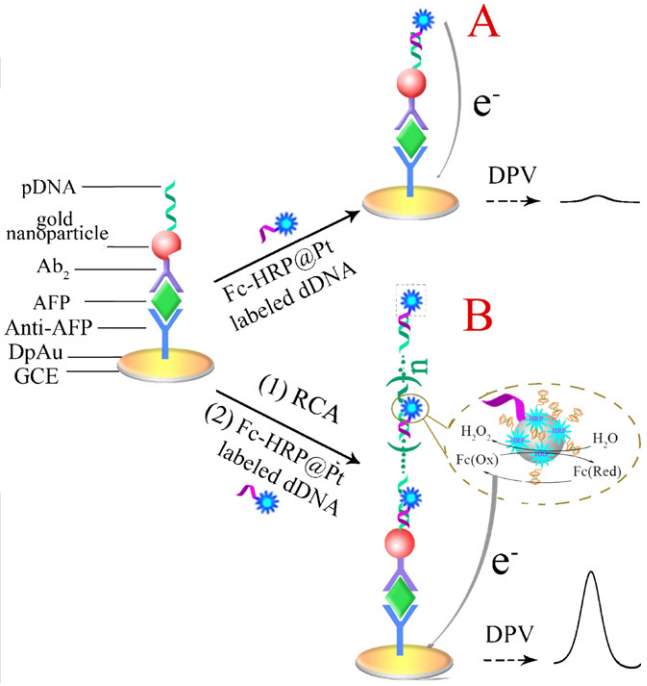
The synthesis of pDNA@Au labeled Ab_2 was briefly by following steps. Firstly, the Ab_2 (100 μg/mL, 100 μL) was added to the Au nanoparticles (approximately 16 nm) solution (2 mL), which were firstly prepared by reducing $HAuCl_4$ with trisodium citrate at 100 °C for half an hour. After 5 h at 4 °C, the mixture was centrifuged and the particles were washed with washing buffer (300 μL, 50 mM, pH 7.5 Tris–HCl buffer and 0.05% Tween 20). Subsequently, 5′-thiol-modified pDNA (10 μM, 50 μL) was mixed with Ab_2 conjugated gold nanoparticles. The solution was allowed to stand overnight at 4 °C. After the final solution was centrifuged, washed with washing buffer and redispersed, the composite of pDNA labeled Ab_2 was obtained, stored at 4 °C when not in use.

2.3. The preparation of Fc–HRP@Pt labeled DNA probe

Firstly, 5′-thiol-modified dDNA (5 μM) were mixed with as-prepared Pt nanoparticles (2 mL) and left overnight under gently stirring. The obtained solution was then added with superfluous HRP, and aged for another 5 h. The HRP@Pt labeled DNA was collected by centrifugation and redispersed in 50 mM, pH 7.4 Tris–HCl buffer. Followed that, superfluous ferrocenemonocarboxylic (Fc), which was activated by EDC and NHS (EDC:NHS = 4:1) for 2 h, was mixed with the HRP@Pt labeled DNA solution. After gently stirred for 12 h, the resulting Fc–HRP@Pt labeled DNA solution were centrifuged and washed three times with washing buffer. The obtained particles were resuspended in 50 mM, pH 7.4 Tris–HCl buffer and stored in 4 °C when not in use.

2.4. Sandwich electro-immunoassay based on RCA-enzyme

Scheme 1 illustrates the detection procedure of the proposed immunoassay. GCE was cleaned by polished with 1.0 and 0.3 μm alumina slurry respectively. Subsequently, it was washed by ultrasonic with bi-distilled water and ethanol for several times, and was dried at room temperature. The capture antibody (anti-AFP) (150 μL) was dropped on the GCE deposited nano-sized gold particles. Then, BSA was utilized to block the remaining bare region by immersing the modified electrode in 0.25% BSA solution for 2 h. Subsequently, various concentrations of AFP analyte were incubated with the immobilized anti-AFP at 37 °C, respectively. After



Scheme 1. Schematic representation of the proposed detection strategy (B). After Ab_2 was captured to the electrode, padlock template was hybridized with the pDNA. Subsequently, phi29 DNA polymerase initiated the RCA with the existence of dNTP. Finally, the Fc–HRP@Pt labeled dDNA probes were linked to the RCA products by base pairing and the electrochemical signal (DPV) could be recorded. Meanwhile, the Fc–HRP@Pt labeled dDNA was directly hybridized with the pDNA as comparison (A).

that, pDNA@Au labeled Ab_2 (50 μL) was dropped to the modified electrode and immune recognized specifically at 37 °C. The dissociative material was removed by washing twice with washing buffer after each step.

The detection procedure could be summarized as following steps: firstly, both ends of the padlock probe were designed to match the sequence of pDNA, so that the padlock probe (50 μL) could hybridize with the pDNA at 37 °C for 30 min. After the padlock probe was circularized by T4 DNA ligase at 22 °C in ligation buffer for 2 h (50 μL, 0.5 U T4 DNA ligase, 50 mM pH 7.5 Tris–HCl buffer, 10 mM $MgCl_2$, 10 mM dithiothreitol, 0.5 mM ATP), the RCA procedure of was initiated by the addition of dNTP (1.0 mM) and phi29 DNA polymerase (0.5 U) in 50 μL reaction buffer containing 50 mM pH 7.5 Tris–HCl buffer, 10 mM magnesium acetate, 33 mM potassium acetate, 1 mM dithiothreitol, and 0.1% Tween 20. The procedure of RCA was carried out at 37 °C. Secondly, a volume of 50 μL Fc–HRP@Pt labeled dDNA probe was applied to hybridize with the immobilized concatameric RCA products because the repeated sequence of RCA products were complementary to the dDNA as designed. The incubation would last 1 h at 37 °C. Then, the prepared electrode was used for electrochemical detection.

2.5. Electrochemical detection

The amperometric responses of the immunosensor were recorded by differential pulse voltammetry (DPV) with a pulse amplitude of 50 mV and a pulse width of 50 ms from 0 to 0.5 V. The cyclic voltammetric measurements were performed from −0.1 to 0.5 V at 50 mV/s. The electrochemical detection was performed in an electrochemical cell containing 0.1 PBS (pH 7.4) and 2.1 mM H_2O_2 at room temperature.