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Electrochemical biosensor for p53 gene based on HRP-mimicking DNAzyme-catalyzed deposition of polyaniline coupled with hybridization chain reaction

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ABSTRACT

In this work, an electrochemical biosensor for p53 gene detection was proposed based on the in situ deposition of polyaniline (PANI) catalyzed by G-quadruplex/hemin horseradish peroxidase- (HRP-) mimicking DNAzyme. G-quadruplex structure was formed through hybridization chain reaction (HCR) amplification strategy. First of all, the target p53 DNA hybridizes with the capture DNA assembled on gold (Au) electrode. Then, the released sequences in capture DNA trigger the HCR between two hairpin DNA probes including one-fourth and three-fourth split- G-quadruplex sequences, resulting in the formation of G-quadruplex in the presence of K^+. After interacting with hemin, HRP-mimicking DNAzyme forms, which catalyzes the oxidation of aniline to PANI with H_2O_2 and leads to a measurable "turn-on" electrochemical signal. The electrochemical response of PANI is dependent on the concentration of target DNA. The constructed analysis platform exhibits a good linear response toward p53 DNA sequence in a wide range of concentration from 1.0 fM to 100 pM with the detection limit of 0.5 fM. In addition, this amplified electrochemical biosensor demonstrates specific recognition capacity to discriminate base-mismatched sequences. Furthermore, it has an excellent performance for target DNA detection in real sample and has a great potential for application in bioanalysis and clinical early diagnosis.

1. Introduction

The p53 gene is one of most important tumor suppressor genes, known as the "guardian of the genome" [1], which can code and express p53 protein to suppress cell malignant transformation [2–4]. The resultant p53 protein can induce cell growth arrest, cell differentiation, cell apoptosis and DNA repair [5,6]. However, p53 gene is the most commonly mutated genes in human tumors, leading to the loss of transcriptional activation potency, which causes infinite proliferation of cell and cancer [7,8]. It has been reviewed that, p53 gene is mutated in about 50% of all human cancers [9,10]. Therefore, sequence-specific analysis of p53 gene is extremely crucial in early screening and diagnosis of cancers. Thus far, various of analytical methods have been employed for sensitive determination of p53 gene including fluorescence [11,12], electrochemistry [13], chemiluminescence [14], electrochemiluminescence [15,16], quartz crystal microbalance [17] and electrophoresis [18]. Among these techniques, electrochemical method has attracted considerable interest because of its characteristics such as simple operation, low cost and fast response.

In order to improve the detection sensitivity, many signal amplification strategies have been developed, including rolling circle amplification (RCA) [19,20], strand displacement amplification (SDA) [21], isothermal exponential amplification [22,23], hybridization chain reaction (HCR) [24,25] and so on [26]. Among them, HCR amplification exhibits great potential in signal amplification due to its unique features [27,28]. Firstly, HCR involves the cascade hybridization between two metastable hairpin DNA probes and does not need any enzyme. Secondly, HCR can be proceeded under mild conditions. Thirdly, HCR amplification achieves high selectivity and sensitivity toward the target. Hence, HCR amplification strategy has been applied in various biosensor for

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biomolecules, such as DNA [29–31], peptides [32], microRNA [33,34] and so on [35–37].

Polyaniline is one of the most important conducting polymers, because of its extraordinary characteristics of high environmental stability, easy preparation, good electro-conductivity and redox properties [38]. In general, PANI can be prepared through electrochemical oxidation and chemical polymerization in a strong acid environment. Fortunately, it has been reported that aniline can be polymerized in mild condition through enzymatic oxidation, such as horseradish peroxidase (HRP) [39–41] and HRP-mimicking DNAzyme [42,43]. Therefore, a variety of electrochemical biosensors have been developed through enzymatic polymerization of aniline. For instance, Gao group [44] proposed an electrochemical biosensor for nucleic acid based on the deposition of PANI catalyzed by HRP. Tang group [42] developed a novel sensing platform for pb^{2+} based on HRP-mimicking DNAzyme-catalyzed deposition of PANI. However, the biosensor for DNA based on enzyme-catalytic polymerization of aniline coupled with signal amplification strategy is few reported [45].

Inspired by the aforementioned works, herein, we developed an electrochemical biosensor platform for target p53 gene based on HRP-mimicking DNAzyme-catalyzed deposition of PANI. In this strategy, two DNA hairpin hairpin probes (HP1 and HP2) that include three-fourths and one-fourth of G-quadruplex sequences were designed. The target DNA was immobilized onto gold (Au) electrode through hybridization with capture DNA. Then, HP1 was unfolded via hybridization with the exposed sequence in capture DNA to trigger the autonomous cross-opening process, which yielded G-quadruplex nanowires. G-quadruplex/hemin HRP-mimicking DNAzyme formed through interaction with hemin. The resulting DNAzyme wires catalyze the oxidation of aniline to PANI, resulting in generation of readable electrochemical signals. Hence, the concentration of target p53 DNA can be transferred to the electrochemical responses of PANI. This electrochemical biosensor exhibits high sensitivity and specificity toward p53 gene. It is expected that this sensing assay holds great promise for detection biomolecules in bioanalysis and clinical biomedicine.

2. Experimental sections

2.1. Reagents and chemicals

Hydrogen peroxide (H_2O_2, 30%, w/v) and aniline were obtained from Chemical Regent Co. (Tianjin, China). Acetic acid and sodium acetate were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). 6-Mercapto-1-hexanol (MCH) and 4-(2-hydroxyethyl)piperazine-L-ethanesulfonic acid (HEPES) were purchased from Sigma. All HPLC-purified DNA oligonucleotides, hemin, dimethyl sulfoxide (DMSO), dithiothreitol (DTT), sodium chloride (NaCl), magnesium chloride ($MgCl_2$) and potassium chloride (KCl) were purchased from Shanghai Sangon Biotechnology Co., Ltd. (Shanghai, China). Aniline was distilled under reduced pressure prior to use. Except aniline, all the regents were used without further purification. Ultrapure water (18.2 MΩ cm) was used throughout the experiments. The sequences of all oligonucleotides are listed in Table S1.

All oligonucleotides were dissolved with 10 mM HEPES buffer (100 mM NaCl, 25 mM KCl, 10 mM $MgCl_2$, pH 7.0) into stock solution and stored at −20 °C. They were diluted with 10 mM HEPES buffer to suitable concentrations prior to use. All the hairpin DNAs were heated to 95 °C for 5 min and then allowed to cool down to room temperature before use. The thiol-modified capture DNA (SH-DNA) was treated by incubating with 0.05 M DTT overnight in the dark to reduce the S—S bonds prior to immobilization.

2.2. Gold electrode treatment and immobilization of capture DNA

First of all, the Au electrode was cleaned by immersion in a freshly-prepared piranha solution (3:1 mixture of concentrated H_2SO_4 and H_2O_2) for 30 min, and then rinsed with deionized water. Subsequently, the Au electrode was polished with 0.05 μm alumina suspension, followed by ultrasonic cleaning in ethanol and deionized water, respectively. Finally, the Au electrode was cleaned with deionized water and dried under nitrogen stream. 10 μL of SH-DNA solution (0.5 μM) was dropped on the Au electrode and incubated for 12 h at 37 °C. Then the electrodes were rinsed with phosphate buffered saline (PBS) (10 mM, pH 7.0) to remove the weakly absorbed capture DNA. After that, the electrodes were immersed into 2 mM MCH for 1 h to block the unoccupied sites of electrodes, followed by washing thoroughly with ultrapure water.

2.3. Target DNA induced hairpin assembly and hybridization chain reaction

The 10 μL of 10 mM HEPES buffer containing different concentrations of target DNA was dropped on surface of the resultant modified Au electrode (resulting from part 2.2) and incubated for 2 h at 37 °C. Afterward, 10 μL of 10 mM HEPES buffer containing 0.5 μM HP1 and 0.5 μM HP2 was dropped on Au electrode surface for HCR for another 2 h at 37 °C.

2.4. Formation of HRP-mimicking DNAzyme and PANI

The resultant electrodes were immersed in 10 mM HEPES buffer containing 4.0 μM hemin and incubated for 1.0 h to form HRP-mimicking DNAzyme. Finally, the electrodes were immersed into 0.1 M HAc-NaAc solution (pH 4.3) containing 30 mM aniline and 4.0 mM H_2O_2, and kept for 2.5 h to perform the deposition of PANI.

2.5. Apparatus

All the electrochemical measurements were performed using a CHI 660D electrochemical workstation (Shanghai Chenhua Instruments Co. Ltd., China). A typical three-electrode system was used. Gold electrode (Au, 2 mm in diameter) was used as the working electrode. A platinum wire electrode and an Ag/AgCl (in saturated KCl) electrodes were employed as the counter and reference electrodes, respectively. Differential pulse voltammetry (DPV) measurements were performed in 0.1 M HAc-NaAc solution (pH 4.3). Electrochemical impedance spectroscopy (EIS) was carried out in 0.1 M KCl solution containing $K_3Fe(CN)_6/K_4Fe(CN)_6$ (1:1, 5.0 mM) within the frequency range of 0.1 Hz to 100 KHz at amplitude of 5 mV. The scanning electron microscopy (SEM) measurements were carried on a QUANTA FEG 250 thermal field emission scanning electron microscope (FEI Co., USA).

3. Results and discussion

3.1. Principle of the assay

This method involves two signal amplification, containing HCR and formation of PANI catalyzed by HRP-mimicking DNAzyme, for ultrasensitive electrochemical biosensor. The working principle is illustrated in Scheme 1. Firstly, the capture DNA containing thiol group is immobilized onto the Au electrode surface by Au-S interaction. When the target DNA is present, it hybridizes with and opens the hairpin structure of capture DNA by a toehold mediated strand displacement reaction. Next, the exposed sequences in capture DNA further hybridize with and open the hairpin structure of HP1 through the toehold mediated strand displacement reaction. Then, the newly exposed sticky sequences (in blue) in HP1