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Sensitive PAT gene sequence detection by nano-SiO_2/*p*-aminothiophenol self-assembled films DNA electrochemical biosensor based on impedance measurement

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Abstract

Nano-SiO_2/*p*-aminothiophenol (PATP) film was fabricated by self-assembly and electrodeposition methods. The immobilization and hybridization of DNA on the nano-SiO_2/PATP film were characterized by cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS). EIS was applied to label-free detection of the target DNA according to the increase of the electron transfer resistance (R_{et}) of the electrode surface after the hybridization of the probe DNA with the target DNA. This DNA electrochemical biosensor showed its own performance of simplicity, good stability, fine selectivity and high sensitivity, and was successfully applied to the detection of the PAT gene sequences by a label-free EIS method. The dynamic detection range was from 1.0×10^{-11} to 1.0×10^{-6} mol/L 20-base sequence of the PAT gene, with the detection limit of 1.5×10^{-12} mol/L. This DNA sensor has a good ability of recognizing single- or double-base mismatched DNA sequence with the complementary DNA sequence.

Keywords: PAT gene; *p*-Aminothiophenol; SiO_2 nanoparticle; DNA; Electrochemical impedance spectroscopy

1. Introduction

Nowadays, a great deal of attention has been paid to the transgenic plants all over the world. Developing reliable methods for detecting and quantifying specific transgene sequences of transgenically modified crops has become a focus topic. DNA electrochemical sensors are more likely to become the accurate, sensitive and rapid detection method for the transgenic plant products. DNA electrochemical sensor is particularly suitable to the research of sequence-specific DNA, the DNA sequence selectivity of which can rapidly detect the DNA sequence with a low cost [1–3]. The most critical step in the fabrication of DNA electrochemical sensors is the immobilization of the DNA probe on the surface of the electrode. The immobilization amount of the DNA probe will directly influence the accuracy, sensitivity, selectivity and service life of the DNA electrochemical sensors.

Self-assembled monolayer (SAM) is an efficient and mature method for the DNA immobilization [4], where gold electrode is commonly used as the basic electrode. An ordered and tightly packed film can be formed via the strong Au–S linkage. Different structures of SAM can be obtained by changing the type and the length of the self-assembly molecule.

In order to enhance the DNA immobilization on the SAM surface, the nanomaterials, especially metal and oxide nanomaterials, such as nano Au, Ag, ZrO_2, TiO_2, Fe_3O_4 and so on, were used [5–7]. Silica nanoparticles have also been effectively applied to the immobilization of various biomolecules and proved to be excellent substrates in the fabrication of biosensors [8,9]. In this paper, we report a novel nano-SiO_2/*p*-aminothiophenol (PATP) SAM for the immobilization and hybridization of DNA, which were characterized by cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS). The results indicated that a few amount of single-stranded DNA (ssDNA) was directly immobilized on the surface of the PATP SAM electrode, while much more ssDNA could be immobilized on the surface of the nano-SiO_2/PATP film electrode. This electrochemical DNA biosensor was used to

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detect the DNA sequences related to PAT gene in the transgenic crops with a label-free method using EIS. PAT gene is phosphinothricin acetyltransferase gene, which is an important screening detection gene of the transgenic plants. The dynamic detection range of the sensor to the PAT gene fragment was from 1.0×10^{-11} to 1.0×10^{-6} mol/L and the detection limit was 1.5×10^{-12} mol/L, which is more than one order lower than our previous reported results [10]. The biosensor also had a good ability for recognizing the single- or double-base mismatched DNA with the complementary DNA sequence of the probe DNA.

2. Experimental

2.1. Apparatus and reagents

Electrochemical experiments were carried out with a CHI 660C electrochemical analyzer (Shanghai CH Instrument Company, China), which was in connection with a Au or modified Au working electrode, a platinum wire auxiliary electrode, and a saturated calomel reference electrode (SCE). The pH values of all solutions were measured by a model pHS-25 digital acidimeter (Shanghai Leici Factory, China).

p-Aminothiophenol (PATP, 90%) and tris(hydroxymethyl) amminomethane (Tris) were purchased from Sigma (St. Louis, MO, USA). Piranha solution (v/v 3:1 concentrated H_2SO_4/30% H_2O_2). $K_3Fe(CN)_6$ and sodium dodecylsulfate (SDS) were purchased from Shanghai Chemical Reagent Company, and $K_4Fe(CN)_6$ from Shanghai Hengda Fine Chemical Reagent Company. All of the chemicals were of analytical grade and solutions were prepared in ultrapure water (resistivity: 15–18 MΩ). An aquapro AWL-0502-1002 P device (Chongqing, China) provided the ultrapure water.

Nano-SiO_2 was prepared with ion exchange method [11]. Concentration of nano-SiO_2 (5–10 nm) in pH 9.10 silica sol is 30%. The density of silica sol is 1.19 g/mL.

Materials for the detection of PAT gene sequences: The 20-base oligonucleotides probe (ssDNA), its complementary sequence DNA (cDNA, target DNA, namely a 20-base fragment of PAT gene sequence, which was selected according to the PAT transgenic sequence), single-base mismatched DNA and double-base mismatched DNA, were synthesized by Beijing SBS Gene Technology Limited Company. Their base sequences are as below:

- *DNA probe (ssDNA)*: 5′-GCC ACA AAC ACC ACA AGA GT-3′.
- *Target DNA*: 5′-ACT CTT GTG GTG TTT GTG GC-3′.
- *Single-base mismatched DNA*: 5′-ACT CTG GTG GTG TTT GTG GC-3′.
- *Double-base mismatched DNA*: 5′-ACT CTG GTG GTG CTT GTG GC-3′.

All oligonucleotide stock solutions of 20-base oligomers (50 μmol/L) were prepared using Tris–HCl solution (5.0 mmol/L Tris–HCl, 50.0 mmol/L NaCl, pH 7.0), which were kept at 4 °C. More diluted solutions were obtained via diluting aliquot of the stock solution with ultrapure water prior to use. The hybridization solution was 2× SSC solution of pH 8.5 including the target DNA. 2× SSC solution was consisted of 0.30 mol/L NaCl and 0.030 mol/L sodium citrate tribasic dihydrate ($C_6H_5Na_3O_7 \cdot 2H_2O$).

2.2. Electrochemical measurements

CV was performed with CHI 660C electrochemical analyzer. Supporting electrolyte solution was 1.0 mmol/L $K_3Fe(CN)_6$ and 1.0 mmol/L $K_4Fe(CN)_6$ (1:1) solution containing 0.1 mol/L KCl. The scan rate was 50 mV/s.

EIS measurements were also carried out in above supporting electrolyte solution with CHI 660C electrochemical analyzer. The ac voltage amplitude was 5 mV and the voltage frequency range was from 10 kHz to 0.1 Hz. The applied potential was 172 mV versus SCE.

The reported result for every electrode in this paper was the mean value of three parallel measurements.

2.3. Procedure

2.3.1. Preparation of PATP/Au self-assembled monolayer

A gold disk electrode was polished with a piece of emery paper, followed with 1.0, 0.3, and 0.05 μm α-Al_2O_3 paste, respectively. After being cleaned ultrasonically in 95% ethanol and acetone for 3 min, respectively, the electrode was chemically etched by being dipped into a piranha solution for 10 min to oxidize impurities and then rinsed with ultrapure water.

Self-assembled monolayer was formed by immersing the bare Au electrode into 5 mmol/L *p*-aminothiophenol solution in ethanol at 4 °C for 9 h. The prepared electrode was then rinsed with ultrapure water, and dried under nitrogen gas.

2.3.2. Preparation of SiO_2/PATP/Au modified electrode

In this work, two methods for the immobilization of nano-SiO_2 on the PATP/Au SAM were compared. One was the electrodeposition of nano-SiO_2 at a constant potential on the PATP/Au SAM. Some oxide nanoparticles, such as TiO_2, ZrO_2, etc., may be electrodeposited on the electrode surface [12]. The negatively charged nano-SiO_2 in a silica sol under the condition of pH 9.10 might be electrodeposited on the PATP/Au surface at +1.1 V. Another was the adsorption immobilization of nano-SiO_2 on the PATP/Au SAM. The PATP/Au electrode was dipped into a silica sol of 30% nano-SiO_2 in pH 7.4 phosphate buffer for several hours. The CV peak-current difference between at the SiO_2/PATP/Au and at the PATP/Au was used for the evaluation. The experimental results indicated that the constant potential deposition at 1.1 V for 600 s could immobilize more nano-SiO_2 on the PATP/Au SAM.

2.3.3. Immobilization and hybridization of DNA

The immobilization of the probe DNA on the nano-SiO_2/PATP/Au was tested by three different methods, namely, by immersing adsorption, by dropping the probe DNA solution on the surface of the nano-SiO_2/PATP/Au and by electrodepositing the DNA probe on the surface of the nano-SiO_2/PATP/Au