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RESEARCH PAPER

DNA Electrochemical Sensor Based on PbSe Nanoparticle for the Sensitive Detection of CaMV35S Gene Sequence

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Abstract: PbSe nanoparticle surface modified with cetyltrimethylammonium bromide (CTAB) was synthesized with a hydrothermal route. PbSe nanoparticle/chitosan (CHIT) composite films were prepared on the carbon paste electrode (CPE) and characterized by SEM and electrochemical methods. The immobilization and hybridization of DNA on the composite films were investigated by electrochemical impedance spectroscopy and cyclic voltammetry (CV). PbSe nanoparticle/CHIT composite matrix has some advantages, such as large ratio surface area, good biocompatibility, and good film-forming ability, and can markedly enhance the immobilization of DNA. Differential pulse voltammetry (DPV) was used to the sensitive detection of the hybridization of DNA with methylene violet (MV) as the indicator. This DNA electrochemical sensor can differentiate the completely complementary DNA sequence and the 2-base mismatched DNA sequence, indicating a good selectivity. The specific sequence related to CaMV35S promoter gene in some transgenic plants was satisfactorily detected using this DNA electrochemical sensor with the detection range from 5.0×10^{-11} to 5.0×10^{-6} M and the detection limit of 1.6×10^{-11} M.

Key Words: PbSe nanoparticle; Chitosan; DNA electrochemical sensor; Differential pulse voltammetry; CaMV35S transgene

1 Introduction

During the last decade, with the quick development of the transgenic plants and more transgenic plant products entering people's daily lives, the security of the transgenic plants has attracted more and more attentions. To understand the safety of food from the transgenic plants and the ecological safety of the transgenic plants, considerable interest has been focused on analytical methods of the accurate, sensitive, rapid, and cheap detection for the products from the transgenic plants. The detection techniques for the DNA hybridization can be used to analyze the transgenic plant products[1,2]. Various techniques including enzymatic method[3], fluorescence spectroscopy[4], quartz crystal microbalance[5], surface plasmon resonance spectroscopy[6], and DNA electrochemical biosensor[7] have been proposed for DNA hybridization detection. Electrochemical method, because of its simplicity, low cost, and high sensitivity, has been developing well recently.

In recent years, nano materials such as PbS, CdS and ZnS have been applied to the fabrication of DNA electrochemical biosensor[8–13] and greatly enhanced the detection sensitivity of DNA. Herein, PbSe nanoparticle surface-modified with cetyltrimethylammonium bromide (CTAB) was synthesized with a hydrothermal route. A DNA electrochemical biosensor was fabricated by using nano PbSe and chitosan (CHIT) composite films with a carbon paste electrode (CPE) as the basic electrode. Eighteen-base sequence-specific of CaMV35S promoter gene existed in many transgenically modified crops was sensitively detected by differential pulse voltammetry (DPV) using this DNA biosensor with an organic dye methylene violet (MV) as the DNA hybridization indicator. Till now, no report is published concerning the fabrication of DNA electrochemical biosensor with nano PbSe and its application for detecting the transgenic gene. The sensitivity of this nano PbSe method has almost no difference with the nano sulfide and anodic stripping voltammetric methods. However, the manipulation of this method with DPV detection is simpler and the reproducibility is better.

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2 Experimental

2.1 Instruments and reagents

The reagents for the nano PbSe preparation, such as Se powder, Cetyltrimethyl ammonium bromide (CTAB), $Pb(CH_3COO)_2{\cdot}3H_2O$, and KBH_4, were commercially available and were all of analytical reagent grade. Chitosan (CHIT, MW-1 × 10^6, 90% deacetylation) and graphite powder were purchased from Beijing Reagent Company. MV was purchased from Shanghai Reagent Company. B-R buffer solution (containing 25.0 mM NaCl, pH 6.0), 2 × SSC (0.30 M NaCl + 0.03 M $C_6H_5Na_3O_7{\cdot}2H_2O$, pH 7.0). Sodium dodecylsulfate (SDS, 0.2% $CH_3(CH_2)_{10}CH_2SO_3Na$). Other chemicals were of analytical grade. All solutions were prepared with ultrapure water.

Table 1 shows the oligonucleotide sequences used in this study, which were synthesized by SBS Genetech Co., Ltd. (Beijing, China). The target DNA (*T*-DNA) was 35S promoter from cauliflower mosaic virus (CaMV35S). The probe DNA (P-DNA) was completely complementary sequence with *T*-DNA. The 2-base mismatched DNA was shown with two-mismatched bases underlined. Noncomplementary DNA was abbreviated as ncDNA.

Cyclic voltammetry (CV) and DPV were carried out with a CHI 830B electrochemical analyzer (Chen Hua Instrument Co., Shanghai, China). The electrochemical impedance spectroscopy (EIS) was carried out with a CHI 750 electrochemical analyzer (Chen Hua Instrument Co., Shanghai, China), the AC voltage amplitude of which was 5 mV and the applied potential was 160 mV with the voltage frequency ranging from 0.1 Hz to 10 kHz. The three-electrode system consisted of the modified CPE as the working electrode, a saturated calomel electrode (SCE) as the reference electrode, and a Pt wire as the counter electrode. The transmission electron microscopic (TEM) images were obtained on Hitachi H-800 transmission electron microscope with an accelerating voltage of 200 kV. The scanning electron microscopic (SEM) image of PbSe/CHIT film was taken using JSM-6700F field-emission SEM (Japan JEOL Instrument Company) with an accelerating voltage of 5.0 kV.

2.2 Experimental methods

2.2.1 Preparation of PbSe nanoparticles

A total of 0.2 g selenium (Se) powder was suspended by 0.1 g KBH_4 in 30 ml water, and then KHSe was prepared. The synthetic process of PbSe nanoparticles is briefly described as follows: 0.25 g CTAB was dropped to 30 ml KHSe solution under stirring for 1 h to form a homogeneous solution. Then, 10 ml solution containing 1.2 g $Pb(CH_3COO)_2{\cdot}3H_2O$ was added to the above solution with stirring for 0.5 h. The total volume was about 40 ml, and the concentration of CTAB was 6.25 g l^{-1}. The solution was transferred to a 50 ml teflon-coat stainless steel autoclave, which was sealed and maintained at 150 °C for 24 h. After cooling to room temperature naturally, the resulting black products were filtered off, washed with distilled water and absolute ethanol several times, and dried in vacuum at 50°C for 6 h.

Table1 Oligonucleotide sequences used in this study

P-DNA	5′-TCT TTG GGA CCA CTG TCG-3′
T-DNA	5′-CGA CAG TGG TCC CAA AGA-3′
2-base mismatched DNA	5′-CGA AAG TGG TCC AAA AGA-3′
ncDNA	5′-GCA TCG AGC GAG CAC GTA-3′

2.2.2 Preparation of PbSe/CHIT solution

Appropriate amount of nano PbSe was dispersed in 1 g l^{-1} CHIT solution (1.0% in acetic acid), and the mass ratio of PbSe:CHIT was 1:2. The mixture was sonicated for 5 min before use.

2.2.3 Preparation of CPE and its modification

The CPE was prepared by thoroughly mixing of graphite powder with paraffin at a ratio of 4/1 (w/w) in an agate mortar at 80°C for 2 h. The homogeneous paste was packed into a glass tube of 4.2 mm diameter. The electrical contact was obtained using a copper wire connected to the paste in the tube. The surface of CPE was smoothed with a weighing paper just before using. Then, PbSe/CHIT composite solution (10 μl) was dropped on the surface of the inverted CPE. The modification (PbSe-CHIT/CPE) was completed after air dried. The thickness of the modified membrane can be adjusted by varying the PbSe-CHIT solution volume. In this experiment, 10 μl PbSe/CHIT solution was used.

2.2.4 Immobilization of P-DNA on modified CPE

The PbSe/CHIT composite modified CPE was inverted. Ten microliter of P-DNA (1.0×10^{-4} M) was dropped onto its surface and air dried. The probe-modified electrode (P-DNA/PbSe-CHIT/CPE) was rinsed with water before hybridization.

2.2.5 Hybridization on the surface of the electrode

A total of 10 μl hybridization buffer solution containing *T*-DNA of different concentrations was dropped onto the P-DNA/PbSe-CHIT/CPE surface. The hybridization process was maintained at 35°C for half an hour. After hybridization, the electrode was washed using SDS to remove the unhybridized DNA. Similar procedures were also carried out by using the two-base mismatched DNA sequence and ncDNA sequence.