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# A PDDA/poly(2,6-pyridinedicarboxylic acid)-CNTs composite film DNA electrochemical sensor and its application for the detection of specific sequences related to PAT gene and NOS gene

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**Abstract**

2,6-Pyridinedicarboxylic acid (PDC) was electropolymerized on the glassy carbon electrode (GCE) surface combined with carboxylic group-functionalized single-walled carbon nanotubes (SWNTs) by cyclic voltammetry (CV) to form PDC-SWNTs composite film, which was rich in negatively charged carboxylic group. Then, poly(diallyldimethyl ammonium chloride) (PDDA), a linear cationic polyelectrolyte, was electrostatically adsorbed on the PDC-SWNTs/GCE surface. DNA probes with negatively charged phosphate group at the 5′ end were immobilized on the PDDA/PDC-SWNTs/GCE due to the strong electrostatic attraction between PDDA and phosphate group of DNA. It has been found that modification of the electrode with PDC-SWNTs film has enhanced the effective electrode surface area and electron-transfer ability, in addition to providing negatively charged groups for the electrostatic assembly of cationic polyelectrolyte. PDDA plays a key role in the attachment of DNA probes to the PDC-SWNTs composite film and acts as a bridge to connect DNA with PDC-SWNTs film. The cathodic peak current of methylene blue (MB), an electroactive label, decreased obviously after the hybridization of DNA probe (ssDNA) with the complementary DNA (cDNA). This peak current change was used to monitor the recognition of the specific sequences related to PAT gene in the transgenic corn and the polymerase chain reaction (PCR) amplification of NOS gene from the sample of transgenic soybean with satisfactory results. Under optimal conditions, the dynamic detection range of the sensor to PAT gene target sequence was from $1.0 \times 10^{-11}$ to $1.0 \times 10^{-6}$ mol/L with the detection limit of $2.6 \times 10^{-12}$ mol/L.


*Keywords:* 2,6-Pyridinedicarboxylic acid; Single-walled carbon nanotubes; PDDA; DNA sensor; PAT gene; NOS gene

## 1. Introduction

The demands for innovative analytical device capable of delivering the genetic information in a fast, simple and cheap manner at the sample source are becoming increasingly important. Electrochemical DNA biosensors offer promising routes for interfacing the DNA recognition and signal transduction elements, and are uniquely qualified for meeting the size, cost and power requirements of DNA diagnostics [1–3].

Nanotechnology has recently become one of the most exciting forefronts in analytical chemistry. A wide variety of nanomaterials, especially nanoparticles with different properties have found broad application in many kinds of analytical methods. Owing to their small size (normally in the range of 1–100 nm), nanoparticles exhibit unique chemical, physical and electronic properties that are different from those of bulk materials, and can be used to construct novel and improved sensing devices, in particular, electrochemical sensors and biosensors. The discovery of carbon nanotubes (CNTs) in 1991 [4] opened up a new era in material science and nanotechnology. Due to its large surface area, wide electrochemical window, flexible surface chemistry and unique property to accelerate electronic transfer, CNTs have been recognized as an ideal nanomaterial to fabricate electrochemical DNA biosensors [5–10]. Usually, CNTs-modified electrodes are fabricated by dispersing CNTs solution onto electrode surface and dried in air. Thus, prepared electrodes are more powerful to transfer electrochemical DNA hybridization signal than bare electrodes. However, the unordered CNTs are always random lying on the electrode surface, and easily peel off from the substrate surface, badly

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depressing the detection reproducibility of the sensors. In order to solve the problems, many novel electrochemical biosensors based on the copolymerization of conducting polymer monomer and carbon nanotubes have received significant interest because the incorporation of CNTs into polymers can obtain new composite materials, which possess the properties of each component with a synergistic effect [11–13]. For instance, Xu et al. [11] synthesized PPy/CNTs hybrid composite onto a glassy carbon electrode (GCE) surface by pyrrole electropolymerization in the presence of carboxylic groups ended multi-walled carbon nanotubes (MWNTs–COOH). During the electropolymerization process, MWNTs–COOH coated with PPy was firmly attached onto the GCE surface. Amino group ended ssDNA ($NH_2$-ssDNA) probe was linked onto the PPy/MWNTs–COOH/GCE by using EDAC, a widely used water-soluble carbodiimide for crosslinking amine and carboxylic acid group.

Recently, a novel technique for ultrathin film assembly has been developed which employed alternate adsorption of oppositely charged polyelectrolytes [14–17]. It is important that a complete charge reversal occurs at the surface after each adsorption step to obtain sufficient adsorption of each polyion. This electrostatic adsorption method is very simple and does not need any label to biomolecules. Therefore, it can overcome the disadvantages of the loss of the biomolecular activity and the complexity of the experiment resulting from the biomolecule label. Poly(diallyldimethyl ammonium chloride) (PDDA) is a linear positively charged polyelectrolyte, which can bind negatively charged phosphate of DNA through electrostatic attraction [18–20].

Over the last two decades, polymers have been proven to be a very suitable matrix for biosensors with fast response time, high sensitivity and great versatility in analytical tools [21,22]. Among them, 2,6-pyridinedicarboxylic acid (PDC) has been used in biosensors with different immobilized biomolecules in its films because of its excellent stability and biocapability [23]. In this paper, PDC and carboxylic group-functionalized single-walled carbon nanotubes (SWNTs) were copolymerized on the GCE surface, forming composite film, which was rich in negatively charged carboxylic group for the electrostatic assembly of cationic polyelectrolyte. The PDC-SWNTs composite film showed characteristics of both components, i.e. the good electron-transfer ability and the large surface area, and the potential synergistic effects. DNA probes with negatively charged phosphate group were attached on the PDC-SWNTs/GCE via PDDA. Results showed that the presented method for electrode modification was simple and efficient, offering a stable and powerful modification layer for immobilizing DNA and transducing nucleic acid hybridization. The electrochemical DNA biosensor was applied to the detection of the PAT gene sequences in the transgenic corn and the polymerase chain reaction (PCR) amplification of NOS gene from the sample of transgenic soybean with methylene blue (MB) as indicator using differential pulse voltammetry (DPV). The dynamic detection range of the sensor to PAT gene target sequence was from $1.0 \times 10^{-11}$ to $1.0 \times 10^{-6}$ mol/L, and the detection limit was $2.6 \times 10^{-12}$ mol/L.

## 2. Experimental

### 2.1. Apparatus and reagents

A CHI 660B electrochemical analyzer (Shanghai CH Instrument Company, China), which was in connection with a glassy carbon-modified working electrode, a SCE reference electrode and a platinum wire counter electrode, was used for the electrochemical measurements. The pH values of all solutions were measured by a model pHS-25 digital acidimeter (Shanghai Leici Factory, China). The PCR amplification was performed by an Eppendorf Mastercycler Gradient PCR system (Germany).

2,6-Pyridinedicarboxylic acid and poly(diallyldimethyl ammonium chloride) were purchased from Sigma (St. Louis, MO, USA). SWNTs were purchased from Shenzhen nanotech. Port Co., Ltd. Both sodium dodecylsulfate (SDS) and methylene blue were obtained from Shanghai Reagent Company and used as received. All the chemicals are of analytical grade and solutions were prepared with doubly distilled water.

Materials for the detection of PAT gene sequences: the 20-base oligonucleotides probe (ssDNA), its complementary DNA sequence (cDNA, target DNA, namely a 20-base fragment of PAT gene sequence, which was selected according to the transgenic sequence of phosphinothricin acetyltransferase gene in transgenic corn), single-base mismatched DNA sequence, double-base mismatched sequence and noncomplementary DNA sequence (ncDNA) 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′;
ncDNA: 5′- CAT GGT TGA TCC GTT CGC TG -3′.

Materials for the PCR amplification of NOS gene sample: one pair of primers (19-base for every primer). Their base sequences are as below:

Primer 1: 5′- ATC GTT CAA ACA TTT GGC A -3′;
Primer 2: 5′- ATT GCG GGA CTC TAT CAT A -3′.

The primer 1 was also used as the probe DNA for the detection of PCR amplification of NOS gene sample. The DNA template for PCR amplification was extracted from transgenic soybean according to the method of plant DNA mini prep kit (Shanghai Academy of Agricultural Sciences).

All oligonucleotides stock solutions of 20-base oligomers ($1.0 \times 10^{-6}$ mol/L) were prepared using Tris–HCl buffer solution (5.0 mmol/L Tris–HCl, 50.0 mmol/L NaCl, pH 7.0), which were stored at 4 °C. More diluted solutions were obtained via diluting aliquot of the stock solution with doubly distilled