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Rapid detection of ssDNA and RNA using multi-walled carbon nanotubes modified screen-printed carbon electrode

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Abstract

A method for rapid sensitive detection of DNA or RNA was designed using a composite screen-printed carbon electrode modified with multi-walled carbon nanotubes (MWNTs). MWNTs showed catalytic characteristics for the direct electrochemical oxidation of guanine or adenine residues of signal strand DNA (ssDNA) and adenine residues of RNA, leading to indicator-free detection of ssDNA and RNA concentrations. With an accumulation time of 5 min, the proposed method could be used for detection of calf thymus ssDNA ranging from 17.0 to 345 $\mu g\,ml^{-1}$ with a detection limit of 2.0 $\mu g\,ml^{-1}$ at 3σ and yeast tRNA ranging from 8.2 $\mu g\,ml^{-1}$ to 4.1 $mg\,ml^{-1}$. AC impedance was employed to characterize the surface of modified electrodes. The advantages of convenient fabrication, low-cost detection, short analysis time and combination with nanotechnology for increasing the sensitivity made the subject worthy of special emphasis in the research programs and sources of new commercial products.

Keywords: Chemically modified electrode; Electroanalysis; Biosensors; DNA; RNA; Carbon nanotubes

1. Introduction

Nanotubes offer significant advantages over most existing materials such as carbon fiber. Carbon nanotubes are of fullerene-related structures that consist of graphite cylinders closed at either end with caps containing pentagonal rings. They have been used as ideal probes of scanning probe microscopy (Wong et al., 1998; Poggi et al., 2002; Stevens et al., 2004) and atomic force microscopy (AFM) (Li et al., 1999; Czajkowsky et al., 2000; Wu et al., 2004) due to their unique properties. The AFM using carbon nanotubes as probes has been applied to investigate DNA or RNA structures and their various functional aspects (Li et al., 1999; Czajkowsky et al., 2000; Hohmura et al., 2000; Woolley et al., 2000; Hafner et al., 2001; Schnitzler et al., 2001; Watanabe et al., 2001, 2003; Woolley and Kelly, 2001; Iwabuchi et al., 2002; Shimotani et al., 2003; Yang et al., 2003; Wu et al., 2004). In view of the excellent properties of carbon nanotubes, they have been used to modify different electrodes for preparation of carbon nanotube nanoelectrode arrays and electrochemical sensing of chemical and biological species (Li et al., 2003b). A series of multi-walled carbon nanotube (MWNT) nanoelectrode arrays prepared by embedding MWNT in a SiO_2 matrix have been developed for ultrasensitive determination of DNA/RNA (Koehne et al., 2003, 2004; Li et al., 2003a). A method for selective and sensitive recognition of complementary DNA by chemically grafting single strand DNA (ssDNA) onto aligned carbon nanotube electrodes has been presented (He and Dai, 2004). The carbon nanotubes (CNT) can amplify DNA or protein recognition and transduction events, which may be used as an ultrasensitive method for electrical biosensing of DNA or proteins (Cai et al., 2003a, 2003b; Wang et al., 2004).

Recently, a label-free or indicator-free DNA hybridization detection has been achieved by impedance (Cai et al.,

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2003a) and chronopotentiometric measurements based on MWNT modified glassy carbon electrode (GCE) (Wang et al., 2003). In this work MWNTs were used to modify screen-printed carbon electrodes (SPCEs) for accumulating ssDNA or RNA samples on electrode surface, and a method for direct electrochemical detection of ssDNA or RNA concentration was developed based on direct oxidation of guanine or adenine residues, which was attributed to MWNT-induced electrocatalytic reaction and interfacial accumulation. The electrocatalytic behaviors of MWNTs have also been demonstrated in studying the anodic oxidation of hydrazine (Zhao et al., 2002) and amperometric sensing of H_2O_2 (Wang et al., 2002b).

The direct amperometric detection of ssDNA or RNA concentration has been performed at mercury (Jelen et al., 1997) and electrochemically modified GCE (Wang et al., 2002a). This work used SPCEs as working electrodes to monitor ssDNA or RNA concentration. The advantages of SPCEs, such as simple and low-cost fabrication and conveniently practical application in detection of biomolecules, have been extensively illustrated (Gilmartin et al., 1995; Hart and Wring, 1997; Wang et al., 1998; O'Halloran et al., 2001). The presence of MWNTs greatly amplified the amperometric signal of guanine or adenine residues. Here a method was developed by combining the advantages of SPCEs with the properties of MWNTs. This method was sensitive, low-cost and rapid, and could directly detect the amount of ssDNA down to 2.0 $\mu g\,ml^{-1}$ and RNA down to 8.2 $\mu g\,ml^{-1}$ with a wide linear range, respectively. The proposed sensors could be prepared in batch and used for DNA or RNA concentration detection in new commercial products.

2. Experimental

2.1. Materials and reagents

Calf thymus DNA and yeast tRNA were obtained from Sigma (USA) and used as received. Multi-walled carbon nanotubes (MWNTs) with diameter of around 36 nm and length of about 10 μm were from Nanjing University (Fig. 1). CH-1 carbon paste and BY2100 silver paste came from Shanghai Bao-yin Electronic Materials Ltd. (China). All other reagents were of analytical reagent grade. All solutions were made up with twice-quartz-distilled water. Native dsDNA was dissolved in 0.01 $mol\,l^{-1}$ Tris–EDTA (TE) buffer (pH 8.0) prior to use. ssDNA was produced by heating a native dsDNA solution in a 100 °C water bath for about 5 min and then rapidly cooling in an ice bath. Since RNA could easily degrade through deoxyribonucleases (RNase), RNA solutions were freshly prepared using the diethylpyrocarbonate (DEPC)-treated water for protecting RNA from RNase (Sambrook et al., 1989; Harwood, 1996). RNA and its solution were stored in ultra-low refrigerator (−70 °C) before use.

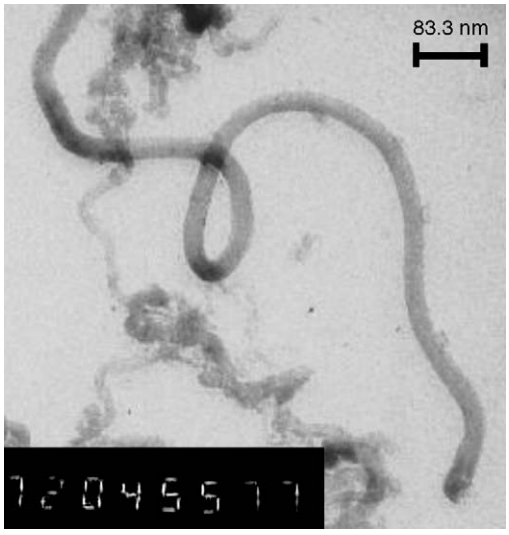


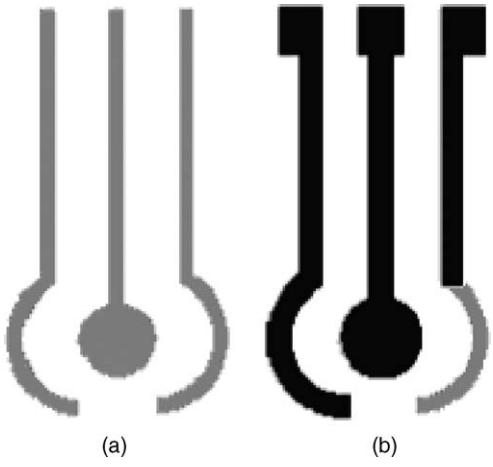
Fig. 1. TEM image of a single multi-walled carbon nanotube (MWNT) (magnification of 120,000:1).

2.2. Preparation of screen-printed electrodes

The combining three-electrode system was printed on a polyvinyl chloride (PVC) membrane (0.025 mm thickness) using a SH/300F screen-printer (Ever Bright Printing Machine Factory Ltd., China). First of all, a sliver base layer (Scheme 1a) was printed on the PVC membrane, then a carbon layer about 0.5–1 mm wider than the silver layer was printed to cover the sliver layer (Scheme 1b). The carbon disc with 4 mm diameter was used as working electrode, the left crescent carbon layer was used as counter-electrode, and the right crescent sliver layer was oxidized electrochemically in 1.0 KCl solution to form an Ag/AgCl reference electrode.

2.3. Modification with multi-walled carbon nanotubes

The ends and surface of carbon nanotubes were treated with concentrated oxidizing acid such as nitric acid to form



Scheme 1.