Alcohol dehydrogenase immobilization on functionalized carbon nano-tubes modified electrode

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ABSTRACT

A thin layer of poly methylen green (PMG) was covered on glassy carbon (GC) electrode surface by electrochemical polymerization method. In the next step by dropping a suspension of carboxylic acid functionalized carbon nano-tubes on the PMG/GC electrode a layer of CNTs was coated on the electrode. Thereafter, to immobilize the enzyme on electrode surface, three layers of PMG, alcohol dehydrogenase and PMG were added to the modified electrode, respectively. The Fourier transform infrared, scanning electron microscopy and cyclic voltammetry measurements clearly confirmed the successful immobilization of enzyme on the GC electrode.

Keywords: Alcohol dehydrogenase; Carbon nanotube; Methylene green; SEM

INTRODUCTION

Generally the enzymes are not very stable in buffer solution [1, 2] but after immobilization their stability is much improved. The duration of stability mostly depends on the method to be used for enzyme immobilization. They include chemical binding, physical adsorption [3], polymer entrapment, encapsulation and cross-linking, etc [1, 2]. Nowadays, there is much interest in the application of various nanostructures as matrix for enzyme immobilization [4-6]. Among the various nanostructures, carbon nanotubes (CNTs), due to their good biocompatibility, high electrical conductivity, high surface area and significant stability are very attractive to be used as host matrix [7-9]. CNTs are cylinder-shaped macromolecules with a radius as small as a few nanometers, which can be grown up to 20 cm in length [10-11]. CNTs exhibit interesting electrical, structural and mechanical properties that make them highly promising nano-scale building blocks for the construction of novel functional materials. Many potential applications have been proposed, such as conductive and high-strength composites, fuel cells, sensors, biosensors and hydrogen storage media [12-14]. In addition, biosensors for detecting abnormalities [15-17] and bio-fuel cells [18] for embedded devices are among the most exciting applications. CNTs can act as electrical connectors between the redox centers of proteins/enzymes and the electrodes. Therefore, they facilitate electron transfer between redox sites of proteins/enzymes and electrode surfaces. In order to create the synergy between the biomolecules and nano-tubes it is necessary to immobilize the biomolecules, such as proteins and DNAs on CNTs. This could be either in the form of non-covalent or covalent bonding. There are several reports concerning the immobilization of biomolecules on CNTs [19-24]. They include proteins such as cytochrome c [25, 26], horseradish peroxidase [27, 28], glucose oxidase [29, 30] and choline oxidase [31].

In the present report alcohol dehydrogenase (ADH), a tetrameric enzyme which catalyses the reversible oxidation of many primary and secondary alcohols [32, 33], besides methylene green were immobilized on functionalized CNTs. The CNT functionalization and ADH immobilization were confirmed and qualified using FTIR, SEM and electrochemical methods.

MATERIALS AND METHODS

Chemicals

ADH (EC 1.1.1.1), β-Nicotinamide adenine dinucleotide (reduced form and disodium salt) (NADH), Potassium dihydrogen phosphate (KH_2PO_4), dipotassium hydrogen phosphate (K_2HPO_4), methylene green (MG), and Poly dially dimethylammonium chloride (PDDA), were obtained from Sigma-Aldrich (St. Louis, MO, USA). CNTs were obtained from Nanotimes Co. (Chengdu, China). All other chemicals were of analytical grade and were used without further purification.

Methods

Fourier Transform Infrared (FTIR) spectra were recorded using a FTIR spectrometer (Thermo Nicolet Co, USA). All Electrochemical measurements were performed using a potentiostat/galvanostat (EG&G, USA). For cyclic voltammetry, a single compartment electrochemical cell (equipped with a platinum rod auxiliary electrode), an Ag/AgCl (saturated KCl) reference electrodes (both from Metrohm), and a glassy carbon (GC) disk (Φ=2 mm) shielded with Teflon as working electrode (from Azar electrode Co., Iran) were used. Scanning electron micrographs (SEM) were obtained using scanning electron microscope (LEO, UK). The sonication of electrode was carried out using ultrasonic bath (Techno-Gaz, Italy).

CNT functionalization

The functionalized CNTs were prepared using acid treatment. Briefly, 10 mg of raw CNTs was dispersed into 10 ml of concentrated nitric acid and sulfuric acid, and then it was sonicated in ultrasonic bath at 45 °C for 8 hours. In the next step, the resultant CNT suspension was filtrated via filter membrane; then, it was thoroughly washed via doubly distilled water as much as the CNTs solution became annihilated. Afterwards, the carboxylic acid functionalized CNTs were collected and dried under infrared lamps. The functinalizations of CNTs were controlled using FTIR spectroscopy and scanning electron microscopy.

ADH immobilization

For ADH immobilization, the GC electrode was cleaned by careful polishing with 1 and 0.3 micron Al_2O_3 to obtain a mirror-like smoothness. Then a thin layer of poly methylen green (PMG) was produced on the GC electrode surface by electrochemically cycling of the GC electrode in 0.4 mM MG solution for 12 cycles. Then the modified electrode was rinsed thoroughly with doubly distilled water to remove any weakly adsorbed MG molecules. In the next step, by dropping 3 µL of functionalized CNT suspension on the PMG/GC electrode, a layer of CNTs was coated on the electrode (CNT/PMG/GC). Thereafter, 2 µL of PDDA was dropped on CNT/PMG/GC and then 3 µL of ADH was absorbed on modified electrode and finally 2 µL of PDDA was dropped on ADH to obtain the designed electrode (PDDA/ADH/PDDA/CNT/PMG/GC).

RESULTS

Figure 1 shows the result of the functionalized process carried out on CNTs. In the FTIR spectrum for raw CNTs (Figure 1, dashed line) three peaks are identified. As seen, a weak band appeared at about 3430 cm^{-1} and two peaks with low intensity at about 1110 cm^{-1} and 1630 cm^{-1}. These peaks are attributed to the presence of -OH, $-CO_2$ and -CO functional groups on the CNTs surface, respectively. The functionalized CNTs (Figure 1, solid line) showed a series of peaks with higher intensity than the raw CNTs. The peaks at 1120, 1630 and 2920 cm^{-1} are attributed to $-CO_2$, -CO and -CH functional groups respectively. Also a strong broad band was observed at 3430 cm^{-1} which is attributed to the –OH functional groups in carbonyl and carboxylic acid.

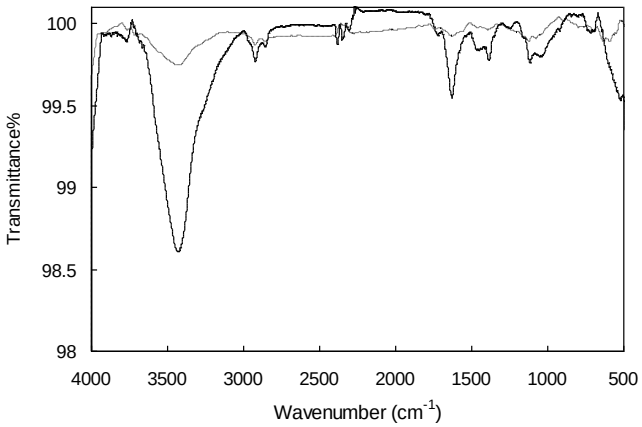

Figure 1. FTIR spectra of raw CNTs (dashed line) and functionalized CNTs by acid treatment (solid line).

Figure 2 shows the SEM images of functionalized CNTs absorbed on GC electrode surface (a), PDDA warped on CNTs (b) and ADH immobilized on PDDA/CNT/PMG/GC electrode surface(c). Figure 2a indicates that the functionalized CNTs have a normal structure. In

addition it shows that the CNT was successfully immobilized on electrode surface. Figure 2.b shows that PDDA polymer warped CNT surface with excellent homogeneity. The homogenous dispersion of CNTs in PDDA polymer could improve the CNT-PDDA composite conductivity on the glassy carbon electrode. Figure 2c, represents how the ADH enzyme is consistently cast on the glassy carbon electrode.

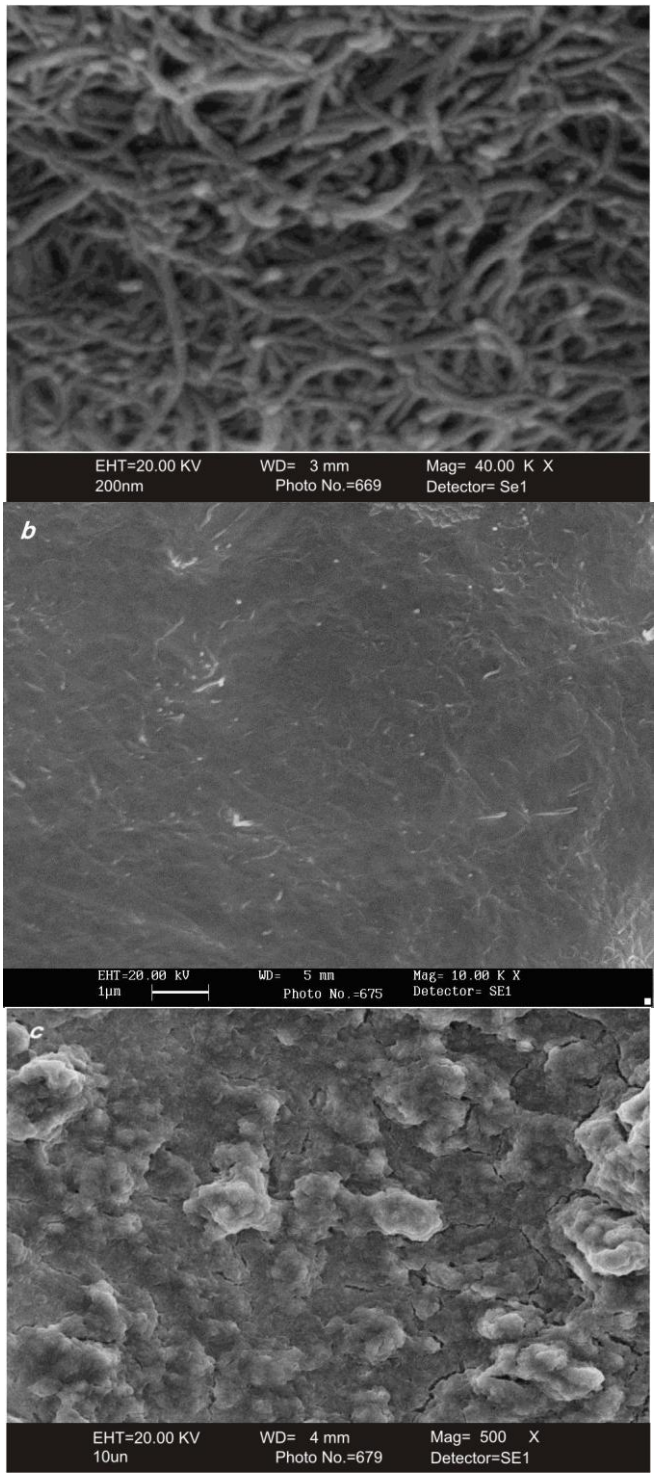

Figure 2. Scanning electron microscopy graphs of functionalized CNTs absorbed on GC electrode surface (a), PDDA warped on CNTs (b) and ADH immobilized on PDDA/CNT/PMG/GC electrode surface(c)

In Figure 3 the cyclic voltammograms of PMG/GC (dotted line), the CNT/PMG/GC (dashed line) and PDDA/ADH/PDDA/CNT/PMG/GC (solid line) electrodes were compared. As shown, after the formation of PMG at the GC electrode surface a very weak and broad redox peak was observed (dotted line). Absorbing the functionalized CNTs on PMG/GC electrode could monitor the redox peak of PMG on the GC electrode (dashed line). This peak indicates the synergic effect of CNTs on strengthening the cyclic voltammogram of PMG. By immobilization of the enzyme through PDDA/ADH/PDDA layers, the height of cyclic voltammogram is reduced (solid line). This is due to the addition of the non-conductive layers of PDDA/ADH/PDDA on high conductive CNTs layer, confirming the successful immobilization of enzyme on the modified electrode.

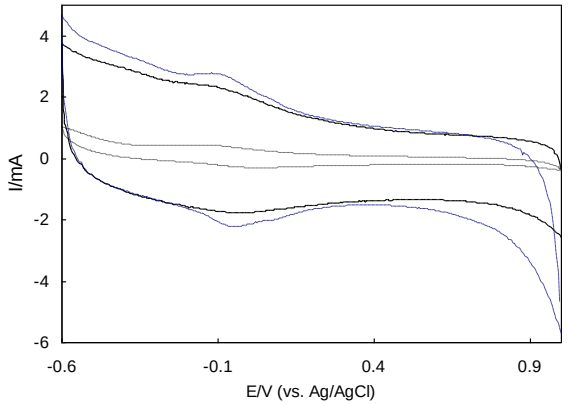

Figure 3. Cyclic voltammograms of different glassy carbon modified electrodes: PMG/GC electrode (dotted line), CNT/PMG/GC electrode (solid line) and PDDA/ADH/PDDA/CNT/PMG/GC electrode (dashed line). All data obtained in 0.1 M phosphate buffer solution, pH 7.4, at 25 ^{o}C. The scan rate was 100 mVs^{-1}

DISCUSSION

Comparison of the FTIR spectroscopic data of the functionalized and raw CNTs revealed that, by acid treatment, a series of carbonyl and carboxylic acid functional groups (in the form of -CO2, -CO, -CH and –OH) were produced on the CNTs.

The SEM images and also the reduced height of cyclic voltammogram confirmed the successful immobilization of enzyme on the modified electrode. The decrease in height of cyclic voltammogram was due to the addition of the non-conductive layers of PDDA/ADH/PDDA on high conductive CNTs layer. Therefore, based on these data one can come to this conclusion that the ADH was successfully immobilized on the modified GC electrode. This approach not only provides an efficient method for immobilizing ADH on functionalized CNTs but also makes the immobilized ADH available for acting as a biological recognition element for the detection of ethanol.

As conclusion, the procedure introduced in the present report indicated that ADH can be immobilized on functionalized CNTs. It seems that this procedure could be used for immobilization of other proteins too. This method also has the potential to be used for development of biosensor for alcohol detection. Further study in this field is under investigation in our lab.

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