Available online at www.sciencedirect.com
ScienceDirect
Biosensors and Bioelectronics 22 (2007) 2768–2773
BIOSENSORS & BIOELECTRONICS
www.elsevier.com/locate/bios

Short communication

Electrocatalytic oxidation of NADH with Meldola's blue functionalized carbon nanotubes electrodes

Liande Zhu*, Jiangli Zhai, Ruilan Yang, Chunyuan Tian, Liping Guo

Department of Chemistry, Northeast Normal University, Changchun, 130024 Jilin, China

Received 4 September 2006; received in revised form 28 November 2006; accepted 20 December 2006
Available online 5 January 2007

Abstract

Meldola's blue (MB) functionalized carbon nanotubes (CNT) nanocomposite film (MB/CNT) electrode was prepared by non-covalent adsorbing MB on the surface of a carbon nanotubes modified glassy carbon electrode (CNT/GCE). Electrochemical behaviors of the resulting electrode were investigated thoroughly with cyclic voltammetry in the potential range of −0.6 to 0.2 V, and two well-defined redox couples were clearly visualized. We also studied the electron transfer kinetics of MB loaded on CNT (MB/CNT) in comparison with that of MB on conventional graphite powder (MB/GP). The heterogeneous electron transfer rate constant (k_s) of MB/CNT was calculated to be about three times larger than that of MB/GP. The accelerated electron transfer kinetics was attributed to the unique electrical and nanostructural properties of CNT supports as well as the interaction between MB and CNT. In connection with the oxidation of nicotinamide adenine dinucleotide (NADH), excellent electrocatalytic activities were observed at MB/CNT/GCE compared with MB/GP modified glassy carbon electrode (MB/GP/GCE). Based on the results, a new NADH sensor was successfully established using the MB/CNT/GCE. Under a lower operation potential of −0.1 V, NADH could be detected linearly up to a concentration of 500 μM with an extremely lower detection limit of 0.048 ± 0.02 μM estimated at a signal-to-noise ratio of 3. Sensitivity, selectivity, reproducibility and stability of the NADH sensor were also investigated and the main analytical data were also compared with those obtained with the MB/GP/GCE.

Keywords: Meldola's blue; NADH; Carbon nanotubes; Graphite powder; Electrocatalysis

---

1. Introduction

Electrochemical oxidation of nicotinamide coenzymes (e.g. nicotinamide adenine dinucleotide, abbreviated as NADH) has attracted a great deal of attention in respect of developing amperometric biosensors, biofuel cells and bioelectronic devices associated with NAD^+ (the oxidized form of NADH) dependent dehydrogenases. Near 300 dehydrogenases are known which require nicotinamide coenzymes as co-reactants. Direct oxidation of NADH at conventional electrodes, such as gold, platinum, and carbon, however, require a large overpotential (>1 V) owing to the sluggish charge transfer kinetics (Moiroux and Elving, 1978; Jaegfeldt, 1980). Numerous efforts have been contributed to accelerating the electron transfer rate between NADH and the conducting substrate by virtue of immobilization of various mediators, which can undergo fairly fast redox reactions with NADH (Jena and Raj, 2005; Grundig et al., 1995; Mano and Kuhn, 1999; Alvarez-Gonzalez et al., 2000; Wu et al., 1996). Recently, some authors claimed that carbon nanotubes based electrodes could lower the overpotential for NADH oxidation (Musameh et al., 2002; Wang et al., 2003a,b,c; Chen and Cai, 2004; Zhang et al., 2004; Wang and Musameh, 2003; Moore et al., 2004). For example, Musameh et al. (2002) observed that the oxidation peak potentials of NADH were 0.33 and 0.36 V for single- and multi-walled carbon nanotubes covered electrodes, which were remarkably negative than that obtained with conventional glassy carbon electrode. Chen and Cai (2004) even found the ordered carbon nanotubes electrode could reduce the oxidation potential near to 0 V. However, the overpotential decrements were greatly varied among literature reports in that different schemes of electrode coating or assembly were employed (Wang and Musameh, 2003; Valentini et al., 2004; Federica and Giuseppe, 2004; Liu et al., 2005; Riccarda et al., 2005). Besides, the electrochemical activities of carbon

---

* Corresponding author. Tel.: +86 431 5099787; fax: +86 431 5269667.
E-mail address: zhuliande@yahoo.com.cn (L. Zhu).

0956-5663/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.bios.2006.12.027

nanotubes are greatly depended on the approaches of synthesis and pretreatment, which would produce different densities of oxygen-related functional groups responsible for catalytic performances, e.g. hydroxyl, carboxyl, and quinone (Musameh et al., 2002; Rao et al., 2001). Another encountered problem was that carbon nanotubes also displayed remarkable catalytic properties towards the oxidation of some other biological substances such as ascorbate, acetaminophen, dopamine, urate, etc. (Pariente et al., 1997), which made carbon nanotubes electrodes difficult to selectively determine NADH in the presence of those compounds. Moreover, the catalytic perspective was still questioned by Compton et al. who compared carbon nanotubes with graphite powder as electrocatalysts for oxidation of NADH, and no superiorities were observed for the carbon nanotubes electrode over graphite powder (Craig and Compton, 2005).

Carbon nanotubes (CNT) consist of seamlessly rolled up graphene sheets of carbon, exhibiting a special sidewall curvature and possessing a π-conjugated structure with a highly hydrophobic surface. These unique properties of CNT essentially allow them versatile to be non-covalent functionalized without destroying their electronic and chemical structures with some organic compounds, in particularly aromatic compounds, through π–π electronic and hydrophobic interactions (Zhang et al., 2003; Li and Zhang, 2004; Basiuk and Rybak-Akimova, 2002; Star et al., 2003). In this paper, Meldola's blue (8-dimethylamino-2,3-benzophenoxzine, abbreviated as MB), a kind of phenoxazine dyes containing naphthaline group which could act as an electron relay for NADH oxidation, selected as a model mediator for NADH oxidation, was firmly assembled on the surface of carbon nanotubes by non-covalent π–π stacking to design a new special nanocomposite interface to stimulate electron transfer with NADH oxidation. The electrochemistry and electron transfer kinetics of the new nanocomposite (MB/CNT) were investigated and compared with those of MB adsorbed on conventional graphite powder (MB/GP). The MB/CNT based electrode also exhibited excellent electrocatalytic activities towards the oxidation of NADH compared with the MB/GP electrode.

2. Experimental

2.1. Reagents

Nicotinamide adenine dinucleotide (NADH) was obtained from Sigma. Single-walled carbon nanotubes (CNT), graphite powder (GP), Meldola's blue (MB), ascorbic acid (AA), acetaminophen (AP), dopamine (DA) and uric acid (UA) were obtained from Aldrich. All other chemicals were analytical grade and used without further purification. Water was purified with a Milli-Q system (Millipore, Bedford, MA).

2.2. Apparatus

Electrochemical measurements were performed with a CHI 660B electrochemical workstation (Chenhua Instruments Co., Shanghai, China). Three-electrode system was employed in this study. The electrodes were inserted into a 10 ml beaker through holes in its Teflon cover. A platinum wire and a Ag/AgCl (3 M NaCl) were used as the auxiliary and reference electrode, respectively. All potentials were referred to the latter. The working electrode was a glassy carbon electrode (diameter: 3 mm, geometric area: 0.0706 cm^2) modified with MB functionalized CNT composite. A magnetic Teflon stirrer provided the convective transport during the amperometric measurements. All the experiments were performed at room temperatures.

2.3. Preparation of MB/CNT film electrodes

CNT were firstly purified by refluxing in concentrated nitric acid for 48 h to eliminate amorphous carbon and metal oxide catalysts, then washed until the pH of the filtrate approached 7 and finally dried at 50 °C in vacuum. With the aid of ultrasonic agitation, 1 mg of the purified CNT was dispersed in 10 ml of *N*,*N*-dimethylformamide (DMF). A glassy carbon electrode was carefully polished with 0.05 μm Al_2O_3 slurry on a polishing cloth and then ultrasonically cleaned in 2 M NaOH, ethanol and distilled water, successively. To modify the glassy carbon electrode with CNT (denoted as CNT/GCE), 10 μl of the CNT suspension was cast onto the surface of the well-polished glassy carbon electrode and the solvent was allowed to dry under an infrared lamp for 30 min.

The MB functionalized CNT electrode was prepared by immersing the CNT/GC electrode in 1 mM MB solution (pH 6.85 PBS) for 10 min. Then, MB could be firmly adsorbed on the surface of the CNT/GC electrode. The prepared electrode (denoted as MB/CNT/GCE) was washed with copious distilled water and then dried in the room temperature. In the control experiment, MB functionalized conventional graphite powder modified glassy carbon electrode (denoted as MB/GP/GCE) was also prepared in the same way.

3. Results and discussion

3.1. Electrochemical behavior of the MB/CNT electrode

Fig. 1(b) depicts the cyclic voltammetric response of the MB/CNT electrode in a pH 6.85 phosphate buffer solution

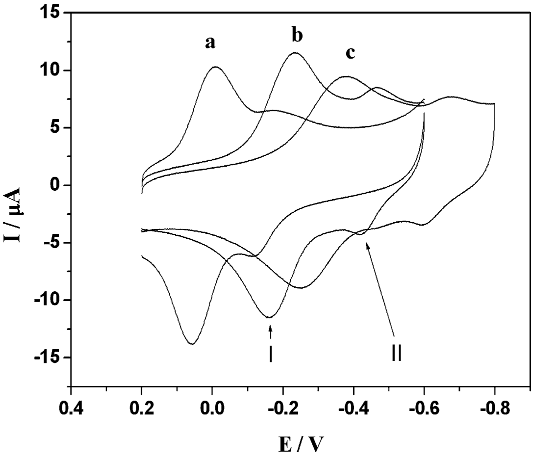

Fig. 1. Cyclic voltammograms for MB/CNT/GCE recorded in 0.05 M phosphate buffer solutions with different pH: (a) pH 2.88, (b) pH 6.85 and (c) pH 10.21. Scan rate: 50 mV/s.