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Direct electron transfer and biosensing of glucose oxidase immobilized at multiwalled carbon nanotube-alumina-coated silica modified electrode

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ABSTRACT

Investigations are reported regarding the direct electrochemical performance of glucose oxidase (GOD) immobilized on a film of multiwalled carbon nanotube-alumina-coated silica (MWCNT-ACS). The surface morphology of the GOD/MWCNT-ACS nanobiocomposite is characterized by scanning electron microscopy. In cyclic voltammetric response, the immobilized GOD displays a pair of well-defined redox peaks, with a formal potential ($E^{\circ\prime}$) of −0.466 V versus Ag/AgCl in a 0.1 M phosphate buffer solution (pH 7.5) at a scan rate of 0.05 V s^{-1}; also the electrochemical response indicates a surface-controlled electrode process. The dependence of formal potential on solution pH indicates that the direct electron transfer reaction of GOD is a reversible two-electron coupled with a two-proton electrochemical reaction process. The glucose biosensor based on the GOD/MWCNT-ACS nanobiocomposite shows a sensitivity of 0.127 A M^{-1} cm^{-2} and an apparent Michaelis–Menten constant of 0.5 mM. Furthermore, the prepared biosensor exhibits excellent anti-interference ability to the commonly co-existed uric acid and ascorbic acid.

1. Introduction

Glucose oxidase (GOD) is a homodimer with a molecular weight of about 150–180 kDa containing two tightly bound flavin adenine dinucleotide (FAD) redox centers embedded deeply in the enzyme [1]. Thus, it is difficult for GOD to carry out a direct electrochemical reaction at an electrode surface because a thick protein layer surrounds its flavin redox center [2]. Many attempts have been made to fabricate heterogeneous direct electron transfer between GOD and underlying electrodes [3–9]. The immobilization of GOD in different types of nanomaterials has been the focus for the development of glucose enzyme electrode, because these nanomaterials could provide a desirable microenvironment between the redox sites of an enzyme and the electrode surface. These methodologies include the immobilization of the GOD on a CdS nanoparticle-modified pyrolytic graphite electrode [6], on an Au nanoparticle-coated microporous Nylon membrane electrode [4], in a nanogold-*N*,*N*-dimethylformamide-ionic liquid composite film [5], on a graphene-chitosan modified electrode [8], and on a graphite nanosheet-Nafion modified electrode [9]. Such surface modifications promote the direct electrochemistry of GOD and facilitate the biosensing application for glucose detection.

Carbon nanotubes (CNTs) possess many unique properties such as high electrical conductivity, high chemical stability, and the ability to promote certain types of electron transfer reactions in electrochemical reactions [10–14]. CNTs have been used for studies on the direct electron transfer of proteins or enzymes because their unique structure and electronic properties allow excellent communication between CNTs and redox active centers of proteins. The CNT-based electrodes have been used to study the direct electron transfer of hemoglobin and horseradish peroxide [15,16]. However, a major problem in using CNTs, especially for biological systems, is the poor dispersability in many solvents because of the large intertube van der Waals interactions. One approach is the functionalization of the surface of CNTs using covalent chemistry, but this can alter the inherent properties of CNTs. Thus, the dispersion of CNTs into suitable medium in noncovalent way to preserve the extended π networks of the CNTs is crucial for electroanalytical applications. In recent studies, an inorganic and noncovalent method to disperse multiwalled carbon nanotubes (MWCNT) in aqueous solutions with alumina-coated silica (ACS) nanoparticle for electroanalytical applications has been demonstrated [17]. The ACS nanoparticles provide new opportunities for biosensing applications because of their large specific surface area and high surface free energy. The MWCNT-ACS nanocomposite has a tendency to self-assemble onto the surface of glassy carbon electrode [18]. It has been shown that the electrochemical properties of CNTs are not impaired by their association with ACS nanoparticles. The combination of MWCNTs and ACS is of great potential to chemical and biochemical areas in both fundamentals and applications.

In this work, the MWCNT-ACS nanocomposite was chosen as the host matrix for the immobilization of GOD which acts as a model redox protein. The GOD was immobilized onto the surface of MWCNT-ACS nanocomposite to form a GOD/MWCNT-ACS nanobiocomposite

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modified glassy carbon electrode. Nafion was coated on the GOD/MWCNT-ACS nanobiocomposite in order to prevent the leakage of GOD. Nafion has been extensively used to fabricate modified electrodes for electroanalysis because of the unique ion exchange, discriminative, and biocompatible properties [19]. The resulting Nafion/GOD/MWCNT-ACS nanobiocomposite film modified glassy carbon electrodes can provide a favorable microenvironment for GOD to perform direct electron transfer and retain its bioactivity. Electrochemical characterization of the Nafion/GOD/MWCNT-ACS nanobiocomposite modified electrode and its subsequent application in the determination of glucose are described. The possible interference behavior is also presented.

2. Experimental

2.1. Reagents

The reagents used were of analytical grade or the highest commercially available purity and were used as received without further purification. GOD (EC 1.1.3.4 from *Aspergillus niger*) and β-D(+)-glucose were supplied from Sigma. 10 mg mL^{-1} GOD solution was obtained by dissolving GOD in 0.1 M phosphate buffer solution (pH 7) and stored at 4°C. Nafion (5 wt.% in mixture of lower aliphatic alcohols and water) was purchased from Aldrich. The 0.5 wt.% Nafion solution used in this work was prepared by diluting the 5 wt.% Nafion solutions into phosphate buffer solution (0.1 M, pH 7). MWCNT (TECO Nanotech Co., Ltd., Taiwan) used in this study was synthesized by an electric arc discharge method and of ~99% purity. The MWCNTs were cylindrical with an inner diameter in the range 2–5 nm, an outer diameter in the range 20–40 nm, and a length of up to several micrometers. Positively charged ACS spheres (Ludox CL) were the product from DuPont. The pH value of the ACS solution was adjusted with 0.1 M HCl. All solutions were prepared with deionized water of resistivity of not less than 18 MΩ cm which was taken from a Milli-Q water purification system (Milli-Q, USA).

2.2. Apparatus

All electrochemical experiments were performed with an Autolab PGSTAT30 Electrochemical Analyzer (Eco Chemie, Netherlands). A conventional three-electrode system was carried out with a 3 mm diameter glassy carbon electrode as the working electrode, an Ag/AgCl (3 M KCl) as the reference electrode, and a platinum wire as the counter electrode (all Metrohm., Switzerland). SEM images were carried out using a JSM-6700F (JEOL, Japan). All potentials were reported with respect to Ag/AgCl (3 M KCl). High purity of nitrogen or air was used for deaeration of 0.1 M phosphate buffer solutions.

2.3. Preparation of Nafion/GOD/MWCNT-ACS nanobiocomposite modified glassy carbon electrodes

Before the surface modification, the 3 mm bare glassy carbon electrode was polished with 0.3 and 0.05 μm alumina slurries and washed with deionized water 3 times. MWCNTs (20 mg) were dispersed in a 1 mL of 1 wt.% ACS aqueous solution (pH 2) with the aid of ultrasonic agitation for 2 h. The self-assembly of MWCNT-ACS onto the surface of glassy carbon electrode was conducted by dipping glassy carbon electrodes into the prepared MWCNT-ACS solution for 1 h. The MWCNT-ACS modified glassy carbon electrodes were rinsed with deionized water 3 times and the solvent was allowed to evaporate at room temperature in the air. The GOD/MWCNT-ACS nanobiocomposite modified glassy carbon electrode was prepared by casting a 6 μL solution of GOD onto the surface of glassy carbon electrode and drying under room temperature in the air. Next, a 6 μL solution of 0.5 wt.% Nafion was coated onto the surface of GOD/MWCNT-ACS. The prepared Nafion/GOD/MWCNT-ACS modified electrode was dried at room temperature and used for electrochemical investigations or stored at 4 °C when not in use.

3. Results and discussion

3.1. Characterization of the GOD/MWCNT-ACS nanobiocomposite by SEM

The surface morphology of the GOD/MWCNT-ACS nanobiocomposite was characterized by SEM in order to compare the MWCNT-ACS nanocomposite and GOD/MWCNT-ACS nanobiocomposite. Fig. 1 shows the SEM images of the MWCNT-ACS before and after cast deposition of GOD. The surface of the prepared MWCNT-ACS (Fig. 1A) nanocomposite was rough and consisted of a great number of ACS nanoparticles. The individual MWCNT was well-dispersed within MWCNT-ACS nanocomposite. MWCNTs would act as high conductivity nanowires connecting nanocomposite domains throughout the MWCNT-ACS nanocomposite. After casting GOD onto the surface of MWCNT-ACS nanocomposite, the surface morphology resulted in a smooth surface, as shown in Fig. 1B. This is attributed to the fact that the GOD was absorbed on the surface of MWCNT-ACS nanocomposite. This result presented that the GOD/MWCNT-ACS nanobiocomposite was uniformly coated on the glassy carbon electrode.

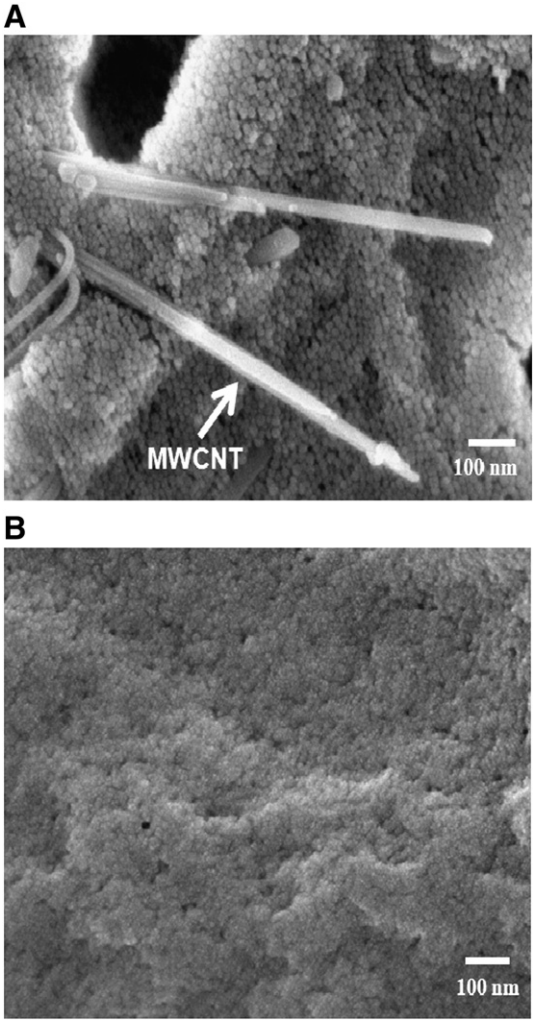

Fig. 1. SEM images of MWCNT-ACS nanocomposite (A) before and (B) after immobilization of GOD.