Chapter 16

Enzymatic Detection Based on Carbon Nanotubes

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Abstract

We describe in this chapter the preparation of simple and cheap carbon nanotube-based biosensor for sensing of glucose. Such biosensor is based on coupling carbon nanotubes and glucose oxidase.

Key words: Carbon nanotubes, Enzyme, Biosensing, Electrochemistry

1. Introduction

The carbon nanotube (CNT) based enzymatic biosensor has attracted considerable attention recently. The CNT can be used as nanowires connecting the redox active center of enzyme with electrode surface, or as electrode material bringing advantages of high conductivity, high surface area, and 3D structure allowing careful nanoarchitectonics of electrodes.

Single-walled carbon nanotubes (SWCNT) can be employed as long-range nanowires, which connect the surface of electrode with the redox center of enzyme (1, 2). As one of the examples, SWCNTs were linked to gold electrode surface by covalent bond. On the other end, they were covalently linked to glucose oxidase redox center (2). The electron turnover rate transferred via SWCNT to the electrode surface was about 4,100 s^{-1}, which is approximately six times higher than the turnover rate of electrons from the active site of GOx to its natural O_2 electron acceptor (700 s^{-1}). Therefore, electron transfer to SWCNT compete favorably with transfer to O_2 and this makes such glucose sensor practically oxygen independent, which is very important in biosensing. In another work, Yu et al. (1) covalently linked enzymes onto the ends of vertically aligned SWCNTs forests (forests are meant in

K. Balasubramanian and M. Burghard (eds.), *Carbon Nanotubes: Methods and Protocols*, Methods in Molecular Biology, vol. 625,
DOI 10.1007/978-1-60761-579-8_16,

this sense arrays of aligned nanoelectrodes), which were used as nanoelectrode arrays. Voltammetry of Fe^{III}/Fe^{II} was observed for the iron heme enzymes myoglobin and horseradish peroxidase attached to carboxylated ends of the nanotube forests by amide linkages. It was suggested that the "trees" in the nanotube forest behaved electrically similar to a metal, conducting electrons from the external circuit to the redox sites of the enzymes.

Carbon nanotube electrodes can incorporate enzymes in various ways. The most straightforward way is to incorporate enzyme in composite material where CNTs are mixed with some polymer binder. Wang et al. (3) combined CNT, Teflon binder, and enzyme and demonstrated that the electrocatalytic activity of CNT toward hydrogen peroxide and NADH permitted effective low-potential amperometric biosensing of glucose and ethanol in connection with the incorporation of glucose oxidase and alcohol dehydrogenase/NAD^+ within the 3D CNT/Teflon matrix. It has been shown that accelerated electron transfer was tied with minimization of surface passivation. The advantages of CNT/Teflon composite devices were demonstrated with comparison to their graphite/Teflon counterparts, which clearly demonstrated higher sensitivity of CNT/Teflon biocomposite.

CNT and glucose oxidase enzyme were incorporated in paste electrodes using oil as binder for glucose biosensing (4) and later on the variety of CNT/paste-incorporated enzymes was expanded towards lactate oxidase, polyphenol oxidase, and alcohol dehydrogenase/NAD^+ (5).

The vast majority of the articles in the area of carbon nanotube-based redox enzyme biosensors incorporate enzyme and carbon nanotubes in the organic or inorganic polymer binder without any chemical bond between enzyme and CNT and/or polymer matrix. Therefore, it is possible not to use polymer binder at all and it is possible to immobilize glucose oxidase on carbon nanotubes by noncovalent binding directly to create CNT-based enzymatic electrochemical glucose biosensor (6).

2. Materials

2.1. Construction of Carbon Nanotube and Graphite Electrodes

1. Carbon nanotubes with small diameter (about 5 nm), i.e., double-walled carbon nanotubes (DWCNT) (Sigma-Aldrich, Germany).
2. Graphite powder, particle size 50 μm (BDH, UK).
3. Nitric acid, diluted to 6 M concentration (Sigma-Aldrich).
4. Glassy carbon electrodes, diameter of 3 mm (Ecochemie, Netherlands).

5. Abrasive paper and alumina paper (polishing strips) (Orion, Spain).
6. X-ray photoelectron spectrometer (XPS) for proof of the adsorption of glucose on carbon surface.

2.2. Chemicals, Analytes, and Electrolytes

1. Glucose oxidase enzyme (GOx) (Sigma-Aldrich).
2. Potassium dihydrogen phosphate and potassium hydrogen phosphate (Sigma-Aldrich).
3. Nitric acid (Sigma-Aldrich).
4. Hydrogen peroxide (30%) (Merck).

2.3. Electrochemical Biosensing

1. Platinum auxiliary electrode (BAS).
2. Double junction Ag/AgCl reference electrode (BAS).
3. Carbon materials (carbon nanotube and graphite) modified glassy carbon electrode as working electrode.
4. Autolab PGSTAT 20 (Eco Chemie, The Netherlands) connected to a personal computer for electrochemical measurements.

3. Methods

3.1. Fabrication of Noncovalently Modified Carbon Nanotube (Graphite) Glassy Carbon Electrodes

1. Purify the carbon nanotubes by stirring them in 2 M nitric acid at 25°C for 24 h. Wash the resulting mixture several times with distilled water until the pH of aqeuos solution reaches neutral pH. Dry carbon nanotubes.
2. Prepare dispersion of carbon nanotubes (0.5 mg/mL) and glucose oxidase (0.2 mg/mL) in distilled water.
3. Stir the solution of carbon nanotubes and glucose oxidase for 75 min.
4. Filter the solution via 0.2 µm membrane.
5. Check if the adsorption of glucose oxidase on carbon nanotube surface was successful using XPS (see Fig. 1). The presence of glucose oxidase will appear as signal for N 1*s*. High resolution XPS will provide confirmatory evidence of presence via strong signal of C–N bond (Fig. 2). High resolution XPS spectrum of DWCNT exhibits tailing peak at 284.5 eV, which is related to sp^2 hybridization of carbon in double-wall carbon nanotube (Fig. 16.2a) (7, 8). The C 1*s* core-level spectrum of DWCNT/GOx shows more complex features (Fig. 16.2b) and it can be curve-fitted with four peak components, with binding energies at 284.5, 285.4, 286.1, and 287.6 eV assigned to the C–H, C–N, C–O and C=O species, respectively (9).

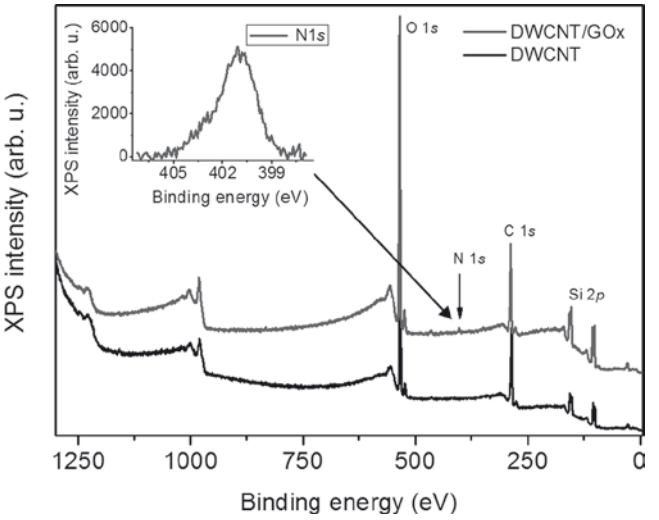

Fig. 1. Wide scan X-ray photoelectron spectrum for DWCNT (black line) and noncovalently functionalized DWCNT/GOx (red line). Also shown detailed spectrum of N 1*s* in DWCNT/GOx sample as inset. Reprinted with permission from (6)

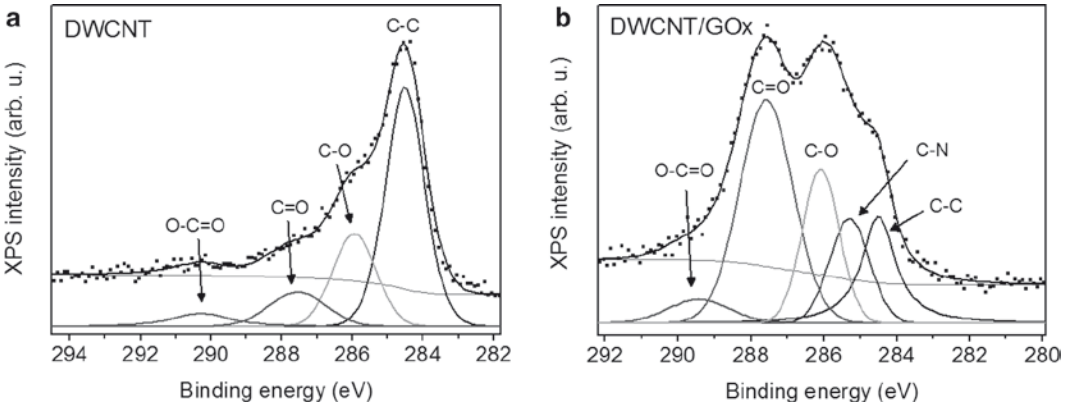

Fig. 2. High-resolution X-ray photoelectron spectrum of C 1*s* core-level spectrum for DWCNT (**a**) and DWCNT/GOx (**b**). Reprinted with permission from (6)

All the above mentioned species are associated with amino acid residues existing in glucose oxidase protein.

6. Disperse the carbon nanotube/glucose oxidase at concentration 1 mg/mL and subject the suspension to ultrasonication for 1 min.
7. Drop the suspension on glassy carbon electrode.
8. Leave it to evaporate the solution to create random network of glucose oxidase modified carbon nanotubes on the surface of the glassy carbon electrode.
9. Repeat procedure 1–8 for graphite instead of carbon nanotubes to have a control material.

3.2. Enzymatic Electrochemical Biosensing Using Carbon Nanotube/Glucose Oxidase Electrodes

1. Glucose oxidase converts glucose to gluconic acid and uses oxygen as the cofactor, which is reduced to hydrogen peroxide as shown in Fig. 3. The hydrogen peroxide is the marker, which is actually detected on the electrode.
2. Prepare supporting electrolyte from phosphate salts. The resulting solution should have concentration of 50 mM and pH of 7.4.
3. Add hydrogen peroxide to the solution and test its electrochemical behavior on carbon nanotube (and graphite)-modified electrode using cyclic voltammetry. The optimal concentration of hydrogen peroxide is about 5 mM.
4. Scan voltage from −200 mV to 1,100 mV with sweep rate of 50 mV s^{-1}. (see Notes 1 and 2).
5. Determine electrochemical activation potential from cyclic voltammograms (for an example of cyclic voltammogram of hydrogen peroxide, see Fig. 4).

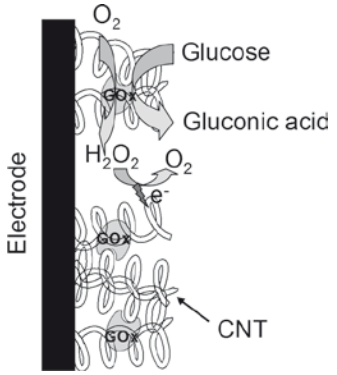

Fig. 3. Enzymatic oxidation of glucose on surface of glucose oxidase-modified carbon nanotubes. Reprinted with permission from (6)

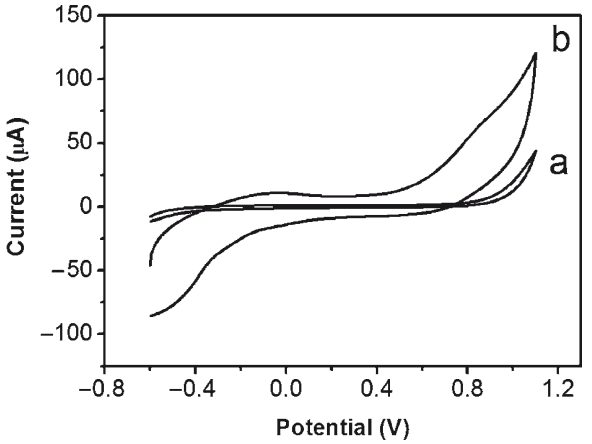

Fig. 4. Cyclic voltammograms for 5 mM hydrogen peroxide using (**a**) graphite/GOx and (**b**) DWCNT/GOx electrodes. Conditions: Scan speed, 50 mV/s; supporting electrolyte, phosphate buffer (50 mM, pH 7.4). Reprinted with permission from (6)

6. Figure 4 presents cyclic voltammograms of hydrogen peroxide (5 mM), which are performed using graphite/GOx (a) and DWCNT/GOx (b) electrodes. It is clearly observed that oxidation of hydrogen peroxide starts around +0.6 V in case of DWCNT/GOx-modified electrode while oxidation of H_2O_2 starts at potential about +0.8 V in case of graphite/GOx-modified electrode. The cyclic voltammograms indicate that DWCNT-based electrode provides high surface compared to graphite and favorable low detection potential for detection of hydrogen peroxide, which is the coproduct of glucose oxidation by glucose oxidase enzyme.
7. Signal of DWCNT electrode towards oxidation of hydrogen peroxide is about 5–6 times higher than the one on graphite electrode. This observation is in line with surface-specific area measurements of DWCNT and graphite measured by BET method and which are 58.55 $m^2 g^{-1}$ for DWCNT and 11.07 $m^2 g^{-1}$ for graphite (about 5× larger surface area of DWCNT than of graphite).
8. The response graphite/GOx and DWCNT/GOx film electrodes toward glucose is scrutinized. Figure 5 presents hydrodynamic voltammograms for 10 mM glucose at the graphite/GOx (**a**) and DWCNT/GOx (**b**) film electrodes. (see Note 3).
9. No significant redox activity is observed for glucose at the graphite/GOx film electrode. This is because glucose oxidase enzyme is not adsorbed on graphite microparticles in large amount.

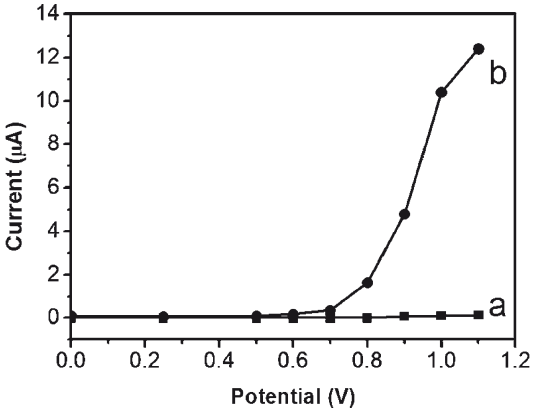

Fig. 5. Hydrodynamic voltammograms for 10 mM glucose working with (**a**) graphite/GOx and (**b**) DWCNT/GOx electrodes. Supporting electrolyte, phosphate buffer (50 mM, pH 7.4); stirring rate, ~550 rpm

10. A significantly different situation prevails in the case of DWCNT/GOx film electrodes. Large oxidation currents, starting around +0.6 V, are observed for glucose at DWCNT/GOx film electrode. The data of Fig. 5 indicate that the GOx redox enzyme was successfully adsorbed on surface of DWCNT. DWCNTs serve as a signal transducer.
11. The outstanding bioactivity of glucose oxidase adsorbed on DWCNT compared to standard graphite is in line with previous work employing enzyme/CNT composites. Asuri et al. (10) demonstrated that CNT–enzyme composites exhibited about 30-times higher overall enzymatic activity than control composites where the proteases were conjugated to nonnanostructure graphite.
12. Stability of the electrode is a fundamental issue. Investigate the stability of immobilized glucose oxidase at DWCNT. Figure 6 demonstrates an example of the amperometric signal of 2.5 mM glucose. No decrease should be observed over time periods in order of 10 min.

4. Notes

1. It is important to note that all electrochemical experiments used deionized water.
2. Solutions during acquiring cyclic voltammogram are static, not steered.
3. Amperometric sensing at fixed potential (i.e., observing stability of electrode) should be carried out in steered solution with magnetic stirrer.

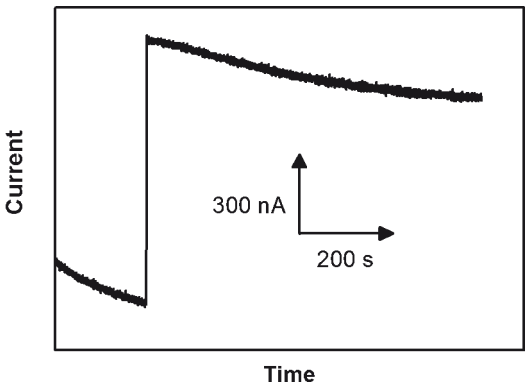

Fig. 6. Response of DWCNT/GOx electrode to 2.5 mM glucose for stability test. Conditions: supporting electrolyte, phosphate buffer (50 mM, pH 7.4); stirring rate, ~550 rpm, detection potential of +0.85 V

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