



Short communication

Electrochemical properties of seamless three-dimensional carbon nanotubes-grown graphene modified with horseradish peroxidase

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ABSTRACT

Horseradish peroxidase (HRP) was immobilized through sodium dodecyl sulfate (SDS) on the surface of a seamless three-dimensional hybrid of carbon nanotubes grown at the graphene surface (HRP-SDS/CNTs/G) and its electrochemical properties were investigated. Compared with graphene alone electrode modified with HRP via SDS (HRP-SDS/G electrode), the surface coverage of electroactive HRP at the CNTs/G electrode surface was approximately 2-fold greater because of CNTs grown at the graphene surface. Based on the increase in the surface coverage of electroactive HRP, the sensitivity to H₂O₂ at the HRP-SDS/CNTs/G electrode was higher than that at the HRP-SDS/G electrode. The kinetics of the direct electron transfer from the CNTs/G electrode to compound I and II of modified HRP was also analyzed.

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1. Introduction

Seamless three-dimensional (3D) sp² carbon-based nanomaterials, such as foam-like graphene [1,2] and carbon nanotubes-grown graphene (CNTs/G) hybrid materials [3,4], have recently attracted great attention not only because of their high electrical conductivity but also their high surface area per unit planar/footprint area. Additionally, since charge carriers are movable in all three dimensions without significant contact resistance, such seamless 3D sp² carbon-based nanomaterials are expected to provide electrode materials for high power fuel cells as well as highly sensitive sensors, compared with seamless 2D sp² carbon-based nanomaterials, such as graphene. Also, because of the flat surface of its backside, the CNTs/G hybrid has the potential for the development of flexible and wearable biosensors for physiological monitoring.

Redox enzymes, such as horseradish peroxidase (HRP) and glucose oxidase (GOx), are widely used for electrochemical biosensors. However, they are generally difficult to directly communicate with flat metal electrodes due to the deeply embedded active site in the thick insulating polypeptide layer. Meanwhile, it is known that CNTs and

graphene flakes can communicate electrochemically with redox enzymes directly, because of their small nanostructure with high conductivity [5–11]. Such carbon nanomaterials have therefore been expected to be utilized for the development of enzyme-based electrochemical biosensors. However, properties of CNTs and graphene emerge only in one direction for CNTs and planar direction for graphene due to their one- and two-dimensional structures, respectively [12–14]. Therefore, controlling the orientation of those carbon nanomaterials is necessary. Additionally, there are also some non-trivial problems, such as the aggregate formation and significant contact resistance among CNTs and graphene flakes.

Toward the development of highly sensitive enzyme-based electrochemical biosensors without the above problems, we previously constructed CNTs/G hybrid electrodes modified with GOx [15] or heme peptide (HP) [16], which has a similar structure to the active center of peroxidase and has often been used for H₂O₂ sensing [17–19]. However, the catalytic activity of HP is lower than that of HRP and therefore an HRP-based biosensor would be more sensitive compared to one based on HP. Thus, there is an interest in using the full HRP for H₂O₂ sensing. In the present work, we constructed HRP-modified CNTs/G electrodes and studied the kinetics of electron transfer from the CNTs/G hybrid to peroxidase. Based on the previous report by Yan et al., we used surfactant sodium dodecyl sulfate (SDS) to immobilize HRP at the CNTs/G [6]. Furthermore, based on the direct reduction of HRP(P)Fe⁴⁺-oxygen complexes (P: porphyrin ring), such as compound I and II, in the presence of H₂O₂ at sufficiently higher potential relative to the formal potential of the HRP(P)Fe^{2+/3+} couple [20,21], we compared catalytic

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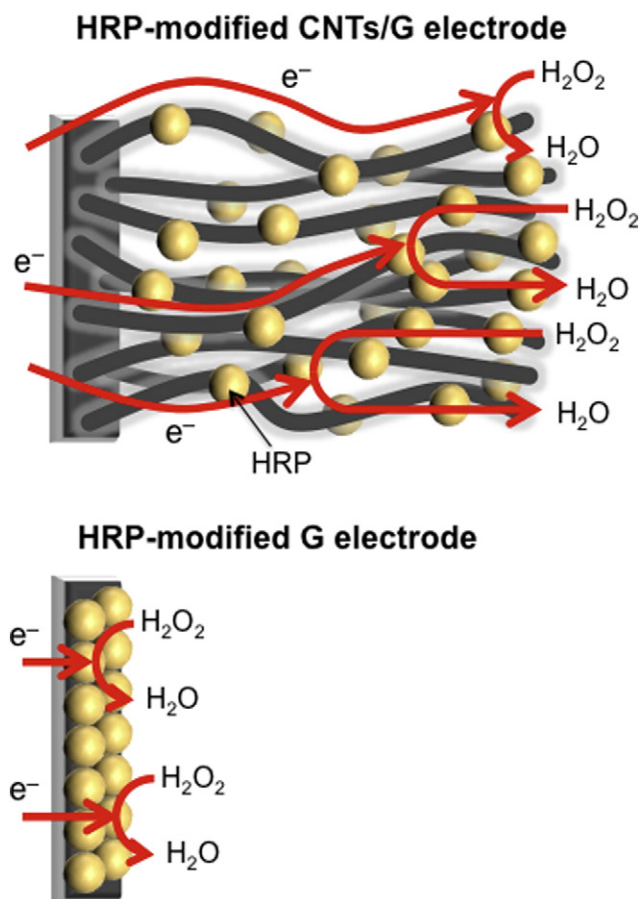


Fig. 1. Schematic illustration of a CNTs/G and G electrodes modified with HRP.

reduction currents of H_2O_2 at the HRP-modified CNTs/G electrode with those at HRP-modified graphene electrode (Fig. 1). Thus, we confirmed the superiority of CNTs/G hybrid films in electrochemical biosensing, compared with graphene films.

2. Experimental

2.1. Preparation of CNTs/G Films

CNTs/G hybrid was prepared according to the previous report [16]. Briefly, a copper (Cu) foil ($\sim 6.5 \text{ cm}^2$ and $25 \mu\text{m}$ thick), which is a catalyst and substrate for graphene growth, was placed inside a quartz tube (ca. 3 cm in inner diameter and ca. 75 cm long) and annealed at $1030 \text{ }^\circ\text{C}$ under flowing Ar (190 sccm) and H_2 (10 sccm) at atmospheric pressure in an electric furnace (Lindberg/Blue M™ Mini-Mite™ Tube Furnace, Thermo Scientific). At this time CH_4 (5 sccm) was introduced in the tube for 9 min to grow graphene followed by cooling of the furnace to room temperature in flowing Ar/ H_2 atmosphere. Subsequently, the graphene-formed Cu foil was decorated with 1.5 nm thick iron film, which acted as catalysts for CNTs growth, using an e-beam evaporator (Temescal BJD-1800, Technical Engineering Services). The graphene/Cu foil with the iron film was placed back in the quartz tube and heated to $750 \text{ }^\circ\text{C}$ in flowing Ar (100 sccm) and H_2 (50 sccm) atmosphere. After the temperature was stabilized at $750 \text{ }^\circ\text{C}$, a 15 sccm flow of C_2H_2 was supplied in the tube for 5 min, followed by cooling of the furnace to room temperature in flowing Ar/ H_2 atmosphere. The graphene film formed on the backside of CNTs/G/Cu foil was removed by O_2 plasma,

followed by etching of the Cu foil in a 1 M FeCl_3 aqueous solution to obtain the CNTs/G hybrid. The CNTs/G hybrid was cleaned with 5% HCl solution to completely remove the Cu foil and the iron nanoparticles. Note that we also obtained the graphene film using the same procedure. Fundamental properties of CNTs/G and graphene films were reported previously [15,16].

2.2. Preparation of HRP-modified electrodes

A glassy carbon (GC) electrode (3 mm in diameter, ALS Co., Ltd.) was polished with 1 and $0.05 \mu\text{m}$ alumina slurries on a polishing cloth and then thoroughly rinsed with distilled water, followed by sonication in 2-propanol and distilled water, respectively. After drying with a high-purity nitrogen stream, the CNTs/G or graphene was transferred to the GC electrode surface and then kept at $50 \text{ }^\circ\text{C}$ in an electric oven for 1 h to be firmly attached on the GC surface. Subsequently, the CNTs/G and graphene electrodes were immersed in either A) pH 7, 67 mM phosphate buffer (PB) containing 0.5 mM HRP (Sigma-Aldrich) with 20 mM SDS for 12 h at $4 \text{ }^\circ\text{C}$ to obtain the HRP-SDS/CNTs/G and HRP-SDS/G electrode, respectively, or B) pH 7, 67 mM phosphate buffer (PB) containing 0.5 mM HRP (Sigma-Aldrich) for 12 h at $4 \text{ }^\circ\text{C}$ to obtain the HRP/CNTs/G and HRP/G electrode, respectively.

2.3. Electrochemical measurements

Electrochemical measurements were performed with a potentiostat Versa STAT (Princeton Applied Research, USA) in 67 mM phosphate buffer (pH 7.4) in a batch system. A Ag|AgCl|KCl (sat.) and a coiled platinum wire were used as reference and counter electrodes, respectively. The catalytic activity of HRP-SDS/CNTs/G and HRP-SDS/G electrodes toward H_2O_2 reduction was evaluated by amperometric measurements. After the working electrode was polarized at $+150 \text{ mV}$ and a steady-state current was obtained, a H_2O_2 solution was added into the electrolyte solution. From the steady-state current obtained here, the catalytic activity of HRP at the CNTs/G and graphene electrodes was determined.

3. Results and discussion

3.1. Electrochemical characterization

Cyclic voltammetry (CV) were performed at the G, CNT/G, HRP/G, HRP/CNTs/G, HRP-SDS/G and HRP-SDS/CNTs/G electrodes in deaerated PB. As shown in Fig. 2A, there was no apparent redox peak at both the CNT/G and graphene electrodes. Similarly, the HRP/CNTs/G and HRP/G electrodes also did not show redox peak (data not shown). On the other hand, redox peaks appeared at both the HRP-SDS/G and HRP-SDS/CNTs/G at approximately -320 mV (vs. Ag|AgCl), corresponding to $(\text{P})\text{Fe}^{2+/3+}$. These findings are similar to reported by Yan et al. [6] and as in their case can be ascribed to properties of the SDS for facilitating protein electrochemistry and the structural properties of CNT and the 3D morphology of the CNT/G electrode. We also evaluated cetyltrimethylammonium bromide (CTAB) for the immobilization of HRP in the phosphate buffer (pH 7.4). However, no redox peaks of $(\text{P})\text{Fe}^{2+/3+}$ were clearly observed. Since CTAB is a cationic surfactant and the isoelectric point of HRP is 8.9, HRP might not be immobilized through CTAB probably due to the electrostatic repulsion between CTAB and HRP. We therefore used only SDS modification for HRP immobilization for further studies.

The redox peak current (j_p) for both electrodes was proportional to the scan rate (ν) below at least 1.5 V s^{-1} (Fig. 2B), indicating that the redox reaction was a surface controlled process. In addition, the redox peak current at the HRP-SDS/CNTs/G electrode was approximately 2-times larger than that at the HRP-SDS/G electrode. This is in accordance with the previously reported ~ 2.5 -fold larger apparent electroactive surface area for the CNTs/G film compared to graphene because of CNTs grown at the graphene surface [16]. Since the peak current was

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