



Electrocatalytic characteristics of electrodes based on ferritin/carbon nanotube composites for biofuel cells

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ARTICLE INFO

Article history:

Received 23 May 2011

Received in revised form 12 July 2011

Accepted 30 July 2011

Available online 6 August 2011

Keywords:

Biofuel cell

Anode

Ferritin

Single wall carbon nanotube

Glucose oxidase

ABSTRACT

A single wall carbon nanotube (SWNT)/ferritin/glucose oxidase (GOx) layer on a glassy carbon electrode acting as a biofuel cell anode was fabricated using an SWNT/ferritin composite as an electron transfer mediator from the enzyme to the electrode. In the absence of glucose, the SWNT/ferritin/GOx composite showed a higher current response than an SWNT/GOx composite, and the electrocatalytic oxidation of glucose on the anode increased linearly with increasing concentration of glucose. The highly distributed SWNT/ferritin composite as a platform for enzyme immobilization resulted in an enhanced electrocatalytic activity towards glucose. The SWNT/ferritin composite showed an enhanced electron transfer from the enzyme to the electrode; therefore, SWNT/ferritin/GOx composites can be used as an anode in biofuel cells.

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1. Introduction

Biofuel cells (BFCs) are fuel cells that generate electrical energy from renewable energy sources via redox reactions under mild conditions. In particular, enzyme-based BFCs have received attention because they are orders of magnitude smaller than equivalently powered microbial cells, allowing operation to take place closer to the redox potential of the enzyme itself [1]. Enzyme based biofuel cell might be applied to implantable medical devices including biosensor, pacemaker, and drug delivery pump. Especially, the composite of ferritin, SWNTs, and glucose oxidase can be possible to use as an anode electrode for biofuel cell system based on this work. It also has the potentials to apply *in vivo* biosensor for detecting blood glucose level.

Because of their outstanding mechanical, geometrical, and transport properties, the applications of single wall carbon nanotubes (SWNTs) have been studied in areas such as biosensors and BFCs for embedded devices [2,3]. SWNTs have a high surface area to weight ratio, as well as the capability to form three-dimensional matrices that can be used for both enzyme immobilization and electrode reactions. However, pristine SWNTs are extremely hydrophobic and prefer to assemble into bundles,

making them difficult to use in homogeneous processes, which limit their application [4]. To overcome the problems encountered with pristine SWNTs, various methods for synthesizing homogeneous SWNT solutions have been studied, such as noncovalent modification using polymers and proteins, and covalent modification using nanobiochemicals. Covalent modification can alter the electronic properties of SWNTs, but it allows for the selective binding of proteins with a higher stability than those achieved using noncovalent modification.

Iron-cored globular-shaped ferritin protein with a diameter of 12 nm has been used in SWNT composites because of its affinity with SWNTs and its multifunctional properties. Ferritin allows for significant ferritin–SWNT conjugation, and increases the dispersion of the nanotubes [5]. Immobilized ferritin on functionalized SWNT composites increases the degree of dispersion of the SWNTs and prevents nanotube aggregation and precipitation [6]. Furthermore, the chemical attachment of ferritin on SWNTs provides novel nanoscale building blocks for the construction of biosensors, biocatalysts, BFCs, and other relevant structures [7]. Previous electrochemical and chemical studies on ferritin have indicated that the protein shell may act as an electron conductor [8], and the mineral core enhances the electronic conductivity of the protein [9]. In particular, ferritin shows redox properties that involve a one-electron transfer per iron atom present that are chemically reversible [10,11].

The combination of the two nanomaterials, ferritin and SWNT, shows synergetic effects that can be used in various applications.

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The natural affinity of nanotubes to the protein increases the degree of dispersion of the SWNTs and the specific capacitance of the composite versus that of pristine SWNT [7]. SWNT/ferritin composites have the potential for use in nano-bio batteries and fuel cells [12,13] and biosensors [6].

In this study, we have fabricated an anode for a BFC based on a ferritin/SWNT composite acting as an electron transfer mediator. The enzyme for oxidation of the fuel, glucose oxidase (GOx), was immobilized on the surface of the SWNT/ferritin composite with a high degree of distribution, and this characterized the electrocatalytic properties of the anode.

2. Experimental

2.1. Chemicals and reagents

The horse spleen ferritin used (85 mg ml^{-1} in 0.15 M NaCl) was obtained from Sigma Chemicals (Milwaukee, USA). The purified SWNTs used were purchased from Carbon Nanotechnologies Inc. (Houston, USA). The sulfuric acid, nitric acid, 1-ethyl-3-(3-dimethylamino propyl)-carbodiimide hydrochloride (EDC), D-(+)-glucose, and phosphate buffered saline (PBS) tablets used were purchased from Sigma Chemicals (Milwaukee, USA). The GOx used, Type X-S from *Aspergillus niger*, was obtained from Sigma Chemicals (Milwaukee, USA). The glutaraldehyde solution used (25% in water) was purchased from Yakuri Pure Chemicals, Japan. The bovine serum albumin used (10 mg ml^{-1}) was purchased from Bioron, Germany, and was used as received. The glassy carbon (GC) electrodes used (MF-2012) were purchased from BAS, USA.

2.2. Preparation of functionalized SWNTs

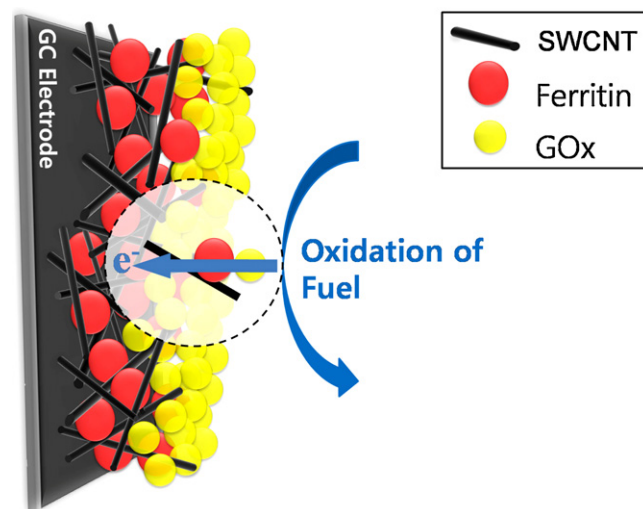
The SWNTs are purified product processed with both gas phase oxidation and acid treatment [7]. As the majority of amorphous carbon, carbon nano particles, and metal catalysts is removed, it reaches the purity of 60–70 wt% (90 vol.%). Diameter is 1.3–1.5 nm, and average bundle thickness is about 20 nm. They were mixed with a solution of sulfuric acid (H_2SO_4 , concentration = 95–98%) and nitric acid (HNO_3 , concentration = 70%) in a 3:1 ratio, and this was sonicated using a bath sonicator (40 kHz, 300 W, Seahan Inc., Seoul, Korea) for a period of 60 min for functionalization. The functionalized SWNTs were then filtered and washed with deionized water until neutral, and then dispersed in water at a concentration of 2.0 mg ml^{-1} .

2.3. Preparation of SWNT/ferritin composites

The SWNT/ferritin composites were prepared using a method described elsewhere [7]. EDC was used for the chemical immobilization of ferritin on the surface of the functionalized carbon nanotubes. Twenty-four milligrams of EDC were added to 2.5 ml of a 2.0 mg ml^{-1} functionalized SWNT solution, and the mixture was stirred for a period of 30 min. Four milliliters of a 5.0 mg ml^{-1} ferritin solution were added to the above mixture, and this was then stirred for a period of 4 h to allow the reaction to complete. After the reaction had ended, the mixture was centrifuged and washed with deionized water twice to remove any excess EDC and unbound ferritin. The SWNT/ferritin composites were dispersed in deionized water for a period of 30 min in a bath sonicator to form the composite solution with a high degree of dispersion.

2.4. Preparation of SWNT/ferritin/GOx electrodes

A GC electrode (diameter = 3 mm) was polished with a $0.05 \text{ }\mu\text{m}$ alumina slurry using a soft synthetic velvet pad. The electrode was cleaned in a bath sonicator for a period of 15 min, and then



Scheme 1. An illustration of the electrocatalytic process on SWNT/ferritin/GOx modified GC electrodes during the oxidation of glucose using ferritin as the electron transfer mediator.

washed with deionized water. After drying the GC electrode, $10 \text{ }\mu\text{l}$ of the SWNT/ferritin composite solution was cast on a GC electrode and dried at room temperature. GOx was dissolved in a PBS solution ($\text{pH} = 5.24$) to maintain the activity of the enzyme during the immobilization process, and $10 \text{ }\mu\text{l}$ of a 10 mg ml^{-1} GOx solution was cast on the dried SWNT/ferritin composite and absorbed. A volume of $10 \text{ }\mu\text{l}$ of bovine serum albumin was cast on the SWNT/ferritin/GOx composite as a carrier protein and absorbed. A volume of $10 \text{ }\mu\text{l}$ of a 1% dilute glutaraldehyde solution was cast on the SWNT/ferritin/GOx composite and absorbed for a period of 5 min. Then, the electrode was dipped in deionized water for a period of 5 min to remove any nonbound GOx, BSA, and glutaraldehyde. The electrode was fully dried, and stored at $4 \text{ }^\circ\text{C}$ until the measurements were performed.

2.5. Characterization

The surfaces of the composite and the composite-based anode were characterized using scanning electron microscopy (SEM). Cyclic voltammetry (CV) and linear sweep voltammetry (LSV) were performed using an eDAQ potentiostat/galvanostat (Model ED401, eDAQ, Australia). The electrochemical experiments were carried out in a three-electrode cell with an Ag/AgCl reference electrode, a platinum wire as the counter electrode, and a GC electrode coated with the SWNT/ferritin/GOx composite film as the working electrode. All electrochemical studies were performed at a temperature of $36 \pm 1 \text{ }^\circ\text{C}$ to characterize the performance of the composite anode for a BFC *in vivo*.

3. Results and discussion

The highly distributed SWNT/ferritin composite was placed on the surface of the GC electrode, and the enzyme, GOx, was immobilized on the surface of the composite. The electrons generated by the enzyme from the oxidation of the fuel, were transferred by the redox reaction of the SWNT/ferritin composite to the electrode, as shown in Scheme 1.

The ability of the composite as an electron transfer mediator placed between the enzyme and electrode was characterized using CV, as shown in Fig. 1(A). The electrochemical properties of the following three samples on the GC electrodes were characterized: (a) GOx, (b) SWNT/GOx, and (c) SWNT/ferritin/GOx. The sample containing GOx on the GC electrode did not show any electrical

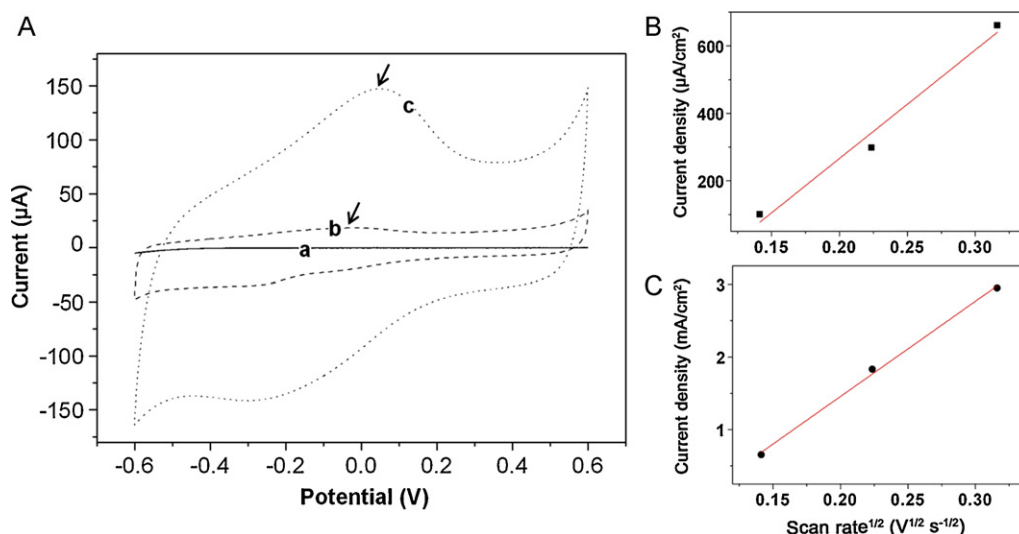


Fig. 1. (A) Cyclic voltammograms of (a) GOx, (b) SWNT/GOx, and (c) SWNT/ferritin/GOx modified GC electrodes in a PBS solution (pH = 7.4), and plots of the peak current density (black arrow) versus the square root of the scan rate of (B) SWNT/GOx and (C) SWNT/ferritin/GOx.

redox reaction with the electrode, because the redox site of the GOx was located inside the structure. When SWNTs were used as the mediator for electrons from the GOx to the electrode, the current response increased by more than two orders of magnitude compared with GOx on the electrode. This indicates that the SWNTs mediated the electron transfer from the enzyme to the electrode. The SWNT/ferritin/GOx composite showed a current response eight times higher than the SWNT/GOx composite. Because the ferritin increases the dispersion of the SWNTs in an aqueous solution, and the SWNT/ferritin composites retain the electrochemical and electronic properties of the ferritin and SWNTs, the efficiency of electron transfer of the composite from the GOx to the electrode increased. In Fig. 1(A), it can be seen that the SWNT/ferritin composites showed an enhanced electron transfer capability versus pristine SWNT. Fig. 1(B) and (C) shows that the potential of the anodic current peaks of SWNT/GOx and SWNT/ferritin/GOx increased linearly with the square root of the scan rate, $v^{1/2}$. This indicates that a diffusion electron transfer process occurred on the surface of the composite. Moreover, it can be seen that the oxidation potential of the SWNT/ferritin/GOx composite on a GC electrode shifted towards a higher potential than the SWNT/GOx composite on a GC electrode because of the immobilized ferritin on the SWNT. It appears that the immobilized ferritin on the SWNTs acted as an electron transfer mediator, resulting in an accelerated electron transfer between the GOx and the electrode [14].

Because the reaction between the fuel and the enzyme occurred on the surface of the electrode, the surface coverage of the enzyme is an important factor determining the electrochemical properties. The surface coverage of the enzyme was characterized using SEM, as shown in Fig. 2. The spherical dots of ferritin were evenly distributed on the electrode from the formation of a composite with the carbon nanotubes, as shown in Fig. 2(A). The surface of the composite was covered by the enzyme, as shown in Fig. 2(B). From a comparison of the SEM images of the SWNT/ferritin composite and GOx on the composite, it is clear that the GOx enzyme was highly distributed on the surface of the composite. Owing to the presence of ferritin, the SWNT was distributed homogeneously on the surface of the electrode, and this gave an effective electron transfer pathway from the enzyme to the electrode.

The electrocatalytic reaction of the SWNT/ferritin/GOx anode towards glucose was characterized with CV using solutions with various concentrations of glucose to evaluate the suitability of SWNT/ferritin/GOx composites as BFC anodes, as shown in Fig. 3. As the concentration of glucose increased from 1 to 40 mM, the electrochemical activity of the composite anodes towards glucose increased, as shown in Fig. 3(A). The position of the oxidation peak of glucose on the SWNT/ferritin/GOx composite shifted from 0.028 to 0.13 V as the concentration of glucose increased. The current response of various samples was in the range 160–195 μA. The current output of the composite anodes versus the concentration of glucose is plotted in Fig. 3(B). The current response increased

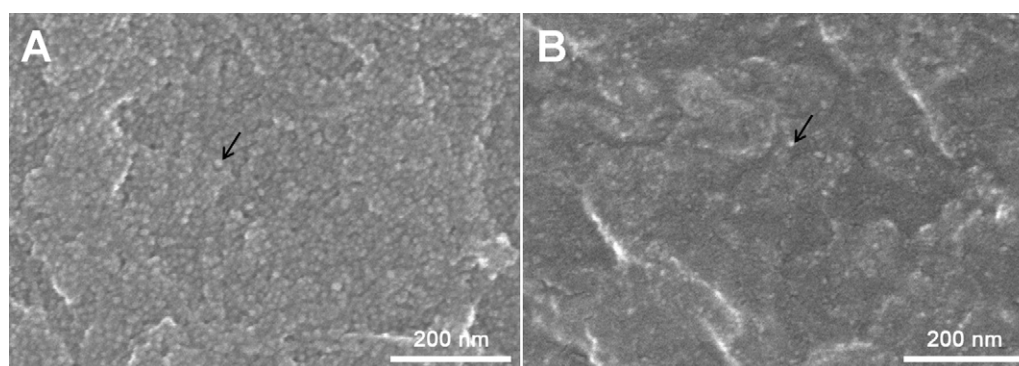


Fig. 2. SEM images of: (A) SWNT/ferritin and (B) SWNT/ferritin/GOx. The spherical dots (black arrow) indicate ferritin. Well-distributed GOx on SWNT/ferritin surface shows as an opaque film to cover spherical dots.

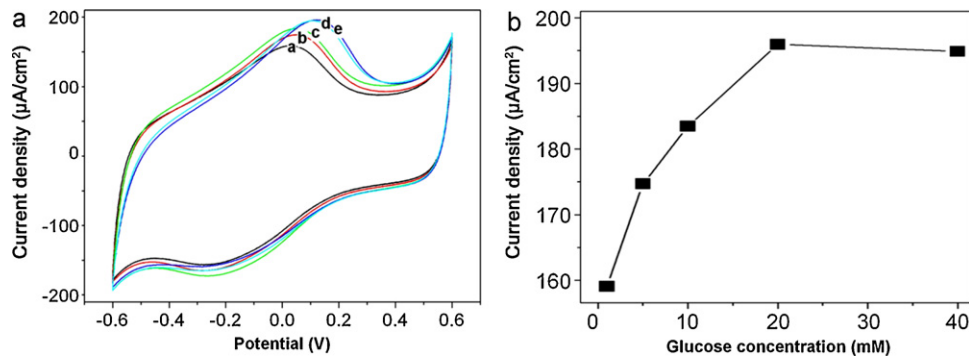


Fig. 3. (A) Cyclic voltammograms of an SWNT/ferritin/GOx modified GC electrode in the presence of glucose at concentrations of: (a) 1, (b) 5, (c) 10, (d) 20, and (e) 40. (B) Dependence of the current output on the concentration of glucose.

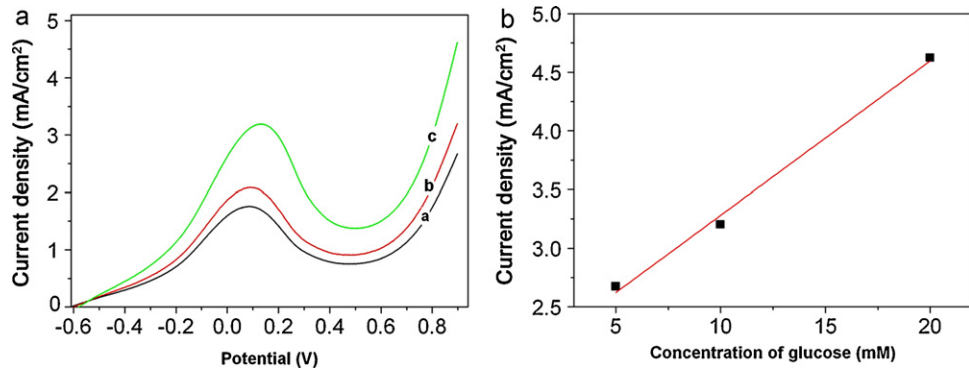


Fig. 4. (A) Linear sweep voltammogram of an SWNT/ferritin/GOx modified GC electrode in the presence of glucose at concentrations of: (a) 5, (b) 10, and (c) 20 mM. (B) The calibration curve corresponding to the electrocatalytic current density versus concentration of glucose.

from 160 μA for 1 mM of glucose to 197 μA for 20 mM of glucose, and this then reached equilibrium at 195 μA for 40 mM of glucose. From the electrochemical data, the surface concentration of SWNT/ferritin/GOx was estimated using the Brown–Anson model [15] based on the following equation:

$$I_p = \frac{n^2 F^2 I^* A V}{4RT},$$

where n is the number of electrons transferred (two in the case of oxidation of glucose by GOx), F is the Faraday constant ($96,584 \text{ C mol}^{-1}$), I^* is the surface concentration of the SWNT/ferritin/GOx composite (in mol cm^{-2}), A is the surface area of the electrode (in cm^2), V is the scan rate (in mV s^{-1}), R is the universal gas constant (in $\text{J mol}^{-1} \text{ K}^{-1}$), and T is the absolute temperature (in K). The surface concentration on the SWNT/ferritin/GOx film on the GC electrode was $1.0 \times 10^{-8} \text{ mol cm}^{-2}$. However, the calculated surface concentration of the enzyme on the electrode using the above equation was 13 times higher than the amount of GOx used ($7.81 \times 10^{-10} \text{ mol cm}^{-2}$). Because the highly distributed SWNT/ferritin composite formed a platform with a nanostructure with a high surface area, there was much more activated GOx on the surface of the electrode. Moreover, the SWNT/ferritin composite acted as an effective electron transfer pathway from the enzyme to the electrode.

The performance of the SWNT/ferritin/GOx modified GC electrode in the presence of different concentrations of glucose (5, 10, and 20 mM) in PBS (pH = 7.4) was characterized using LSV, as shown in Fig. 4(A). The LSV showed that an electrocatalytic oxidation current was generated by the composite electrode. The electrocatalytic activity of the anode towards the oxidation of glucose increased linearly with increasing concentration of glucose. The respective calibration as a function of glucose concentration versus the current density showed that a saturation current density was achieved

at 4.6 mA cm^{-2} at a scan rate 50 mV s^{-1} during the oxidation of a 20 mM glucose solution. Fig. 4(B) shows that the electrocatalytic oxidation current increased proportionally with increasing concentration of glucose between 5 and 20 mM, indicating the enzyme catalyzed the oxidation of glucose on the SWNT/ferritin/GOx modified GC electrode.

4. Conclusions

Chemically immobilized ferritin on the surface of SWNT was used as an electron transfer mediator between GOx and a GC electrode. The ferritin increased the dispersion and current response of the SWNT, and the immobilized ferritin on the SWNT acted as an effective electron transfer pathway, resulting in an enhanced electron transfer from the enzyme to the electrode. Moreover, the highly distributed SWNT/ferritin composite formed a platform with a high surface area for enzyme immobilization. The SWNT/ferritin/GOx anode showed a linear electrocatalytic activity towards glucose with increasing glucose concentration. The calculated activity of the enzyme showed a much higher value than expected because the GOx was highly distributed and wired to the surface of the SWNT/ferritin composite. BFC anodes made from SWNT/ferritin composites could be used for the development of BFCs with a high performance.

Acknowledgement

This work was supported by the Creative Research Initiative Center for Bio-Artificial Muscle of the Ministry of Education, Science and Technology (MEST) in Korea.

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