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Carbon-decorated ZnO nanowire array: A novel platform for direct electrochemistry of enzymes and biosensing applications

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ABSTRACT

We report here the direct electron transfer of GOD and a novel glucose biosensor based on carbon-decorated ZnO(C–ZnO) nanowire array electrode. The C–ZnO nanowire array provides a novel platform for fast direct electrochemistry of GOD, and its based biosensor shows very high sensitivity and low detection limit. Based on the direct electrochemistry of horseradish peroxidase (HRP), the H_2O_2 biosensing application is further demonstrated using this new C–ZnO array architecture. The high conductivity of carbon and good electron transfer capability of ZnO nanowires, along with their low cost and biocompatibility make the C–ZnO nanowire array a promising platform for direct electrochemistry of enzymes and mediator-free enzymatic biosensors.

1. Introduction

Electron transfer of enzymes with electrodes is the essential prerequisite for the development of biofuel cells [1] and protein-based electrochemical biosensors [2]. It can be realized by the use of redox mediators, but has critical limitations [3–5]. As a better approach, direct electron transfer of an enzyme with the electrode, a direct electrochemistry can render high energy conversion efficiency in biofuel cells by its reversible nature while low interference in electrochemical biosensors from its low redox potential [6]. Unfortunately, most enzymes have difficulty to enable the direct electrochemistry at a bare electrode surface, because of their insulation-shelled redox centers [2,7]. Direct electron transfer has been reported only for about 50 out of 1060 redox enzymes known today [6].

ZnO is a very economic functional material [8,9]. It can be used to immobilize enzymes for biosensors due to its high electrochemical activity and biocompatibility [4,10–12]. In particular, as compare to nanoparticles, ZnO nanowires, especially in the array form, possess much better electron transportation capability due to its one-dimensional (1D) feature [4,10]. Direct electron transfer of some enzymes such as microperoxidase, cytochrome C was investigated previously on ZnO [13–16]. However, no report on the direct electrochemistry of glucose oxidase (GOD) at ZnO-based electrode has been demonstrated.

Nanostructured electrodes have been adopted as a promising way to facilitate the direct electron transfer of proteins. These nanostructures include, for example, carbon nanomaterials [17,18], Au nanoparticles [19], and metal oxides [20–22]. Among them, carbon is an important versatile material due to its biocompatibility, high electrical conductivity and large surface area [17]. High conductivity could play an important role in the direct electrochemistry.

We previously reported that ZnO nanowire arrays can be grown directly on bulk electrode substrates [23], enabling robust mechanical adhesion and electrical contact of every nanowire to the substrate. Considering that conventional electrodes were almost fabricated by post-pasting of materials [4,17–22], it is a great challenge to directly grow nanostructure array electrodes for biosensors. Here, we design a new nanowire array electrode by effectively taking advantage of the conductivity and chemical stability of carbon and the 1D channel structure of ZnO nanowires. The electrode, fabricated simply by coating a thin layer of carbon on the surface of ZnO nanowire array via carbonization, can be

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an inexpensive and high-performance alternative to conventional carbon nanotube/Au film electrodes for use in biosensors.

2. Experimental

Pristine ZnO arrays on Ti substrate were prepared as described in [23]. To fabricate carbon-decorated(C–ZnO) nanowire array, the prepared pristine ZnO arrays were immersed in a 50 mL 0.5 M glucose aqueous solution for 10 h. Then the substrate was taken out, dried at 60 °C and further annealed in Ar gas at 500 °C for 4 h. A carbon shell was formed on the nanorod surface without observed nanoparticle or aggregates. This could be ascribed to a uniform thin layer of glucose molecules adsorbed on the nanowire surface due to the good hydrophilicity of ZnO.

For protein immobilization, 10 μL GOD/horseradish peroxidase (HRP) solution (15 mg mL^{-1}, 0.01 M PBS, pH 7.0) was dropped onto 0.5 × 0.5 cm^2 C–ZnO array Ti electrode surface. After the evaporation of water, the electrode was stored at 4 °C for one day. Prior to the measurement, the electrode was rinsed in 0.01 M PBS to remove the unimmobilized GOD/HRP, a 5 μL 0.5 wt.% Nafion solution was further introduced to form a tight membrane on the surface. The electrochemical properties were examined with a dual channel electrochemical workstation (CHI) utilizing a conventional three-electrode system, which consists of a Pt wire as the counter electrode, a saturated calomel electrode (SCE) as the reference, and a C–ZnO nanowire array on Ti as the working electrode. The electrolyte was 0.1 M pH 7.0 PBS solution.

The array product was characterized using powder X-ray diffraction (XRD, Bruker D-8 Avance), transmission electron microscopy (TEM, JEM-2010FEF), scanning electron microscopy (SEM, JSM-6700F), and Raman spectroscope (Witech CRM200, 532 nm). Nitrogen adsorption–desorption measurements were conducted at 77.35 K on a Micromeritics Tristar 3000 analyzer. The BET surface areas were estimated using adsorption data.

3. Results and discussion

As shown in Fig. 1a, the C–ZnO nanowire array has the similar morphology to the pristine ZnO array [23]. The nanowires are typically ~5 μm in length and spaced apart sufficiently. Peaks in XRD pattern (Fig. 1b) can be well indexed to *c*-axis oriented ZnO except for those arising from Ti substrate. Fig. 1c displays the Raman spectrum of the array, which shows two strong peaks located at 1340(D-band) and 1588 cm^{-1}(G-band) in addition to the characteristic peak of ZnO at 438 cm^{-1}. The broad D-band as well as the shift of G-band to high wavenumber with respect to the spectrum of pure graphite crystals (1575 cm^{-1}) suggests the low degree of graphitization [24]. By TEM observations, we can find in most cases that ZnO nanowire is decorated by a thin shell of carbon (~12 nm) (Fig. 1d). The HRTEM image of the shell reveals the disordered lattice planes of carbon.

The detection of glucose levels in blood is of great importance for diagnosis and therapy of diabetes [20,25]. Cyclic voltammogram (CV) of Nafion/GOD/C–ZnO nanowire array electrode is shown in Fig. 2a. This electrode exhibits a pair of well-defined redox peaks at −0.43 and −0.48 V, resulted from the direct electron transfer of the immobilized GOD [17,20]. By contrast, no peaks are observed at both Nafion/GOD and Nafion/C–ZnO nanowire array modified electrodes. Meanwhile, only very weak peaks are detected for Nafion/GOD/pristine nanowire array electrode. Apparently, the carbon deposited on the nanowire surface plays a crucial role in the direct electron transfer. The impedance results

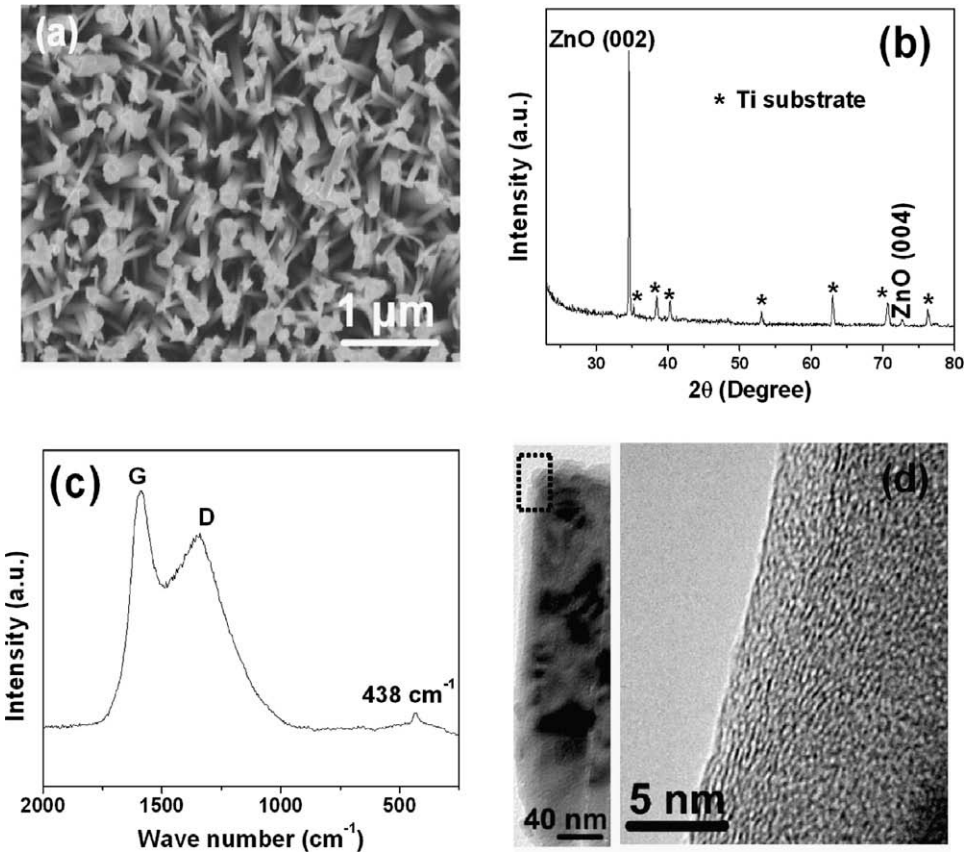

Fig. 1. (a) Typical SEM image of the C–ZnO nanowire array. (b) XRD and (c) Raman spectrum of the array. (d) TEM and HRTEM images of an individual nanowire.

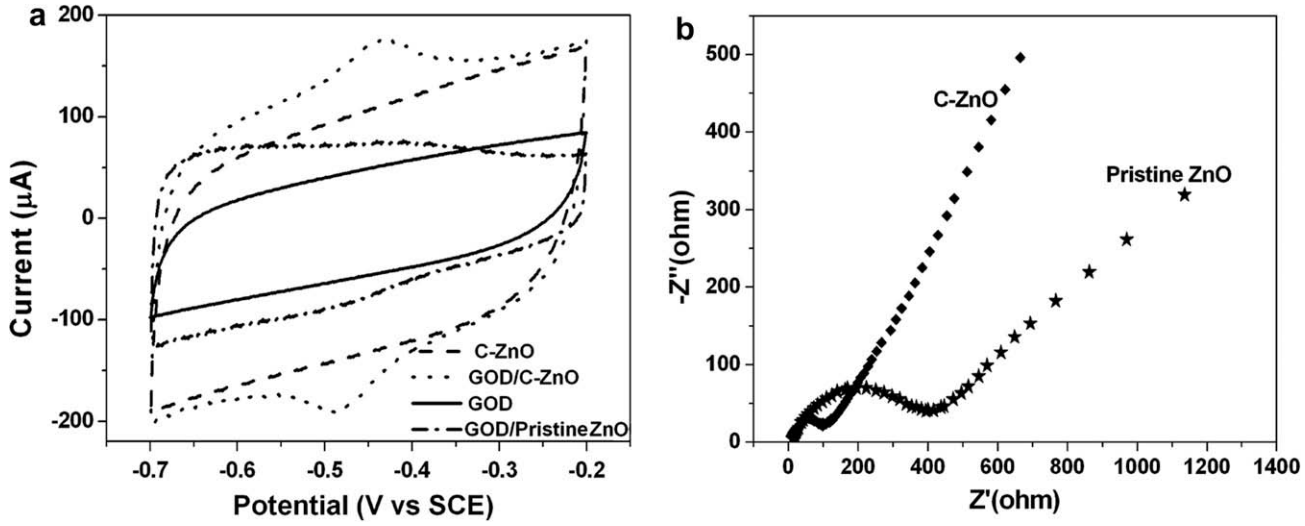

Fig. 2. (a) CVs of different electrodes in N_2-saturated PBS solutions at $0.5\,Vs^{-1}$: Nafion/C–ZnO; Nafion/GOD/C–ZnO; Nafion/GOD; Nafion/GOD/Pristine ZnO. (b) Electrochemical impedance spectroscopy measurements of 10 mM $[Fe(CN)_6]^{3-/4-}$ in 1.0 M KCl using C–ZnO and pristine ZnO electrodes.

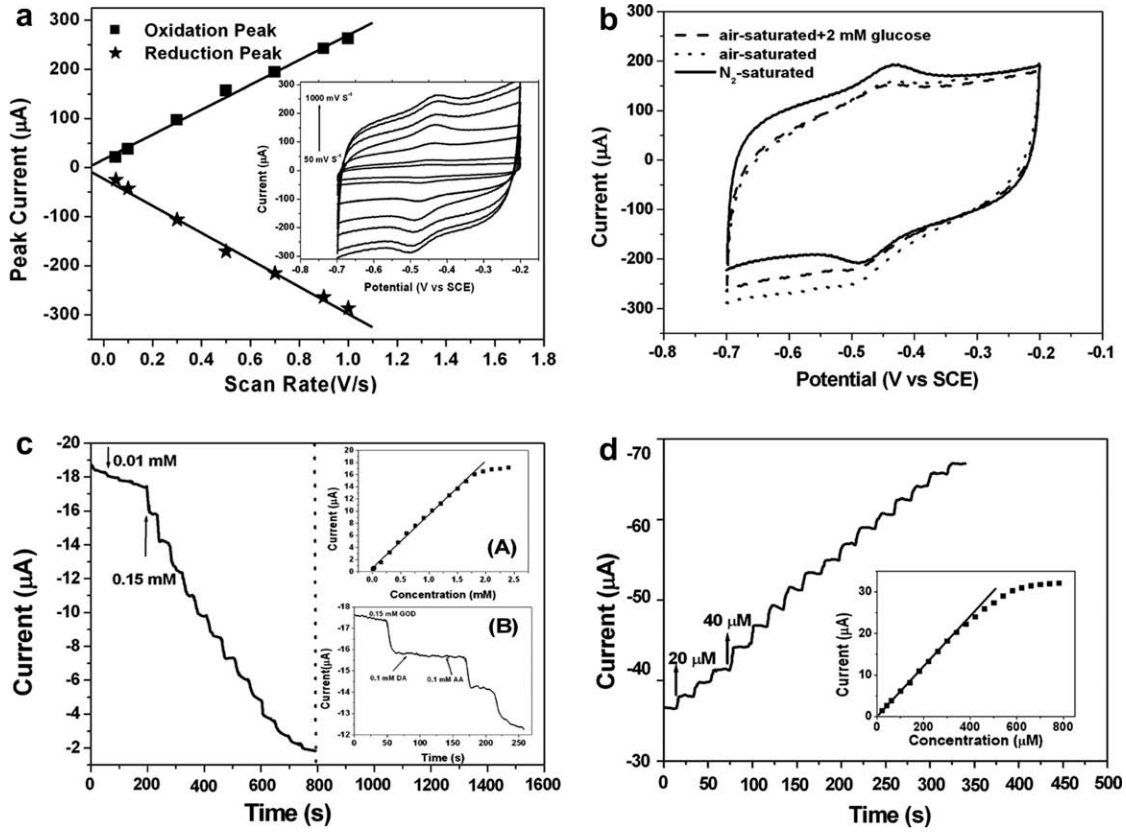

Fig. 3. (a) CVs of Nafion/GOD/C–ZnO electrode in N_2-saturated PBS solution at different scan rates. Inset shows the plots of peak currents versus scan rate. (b) CVs of the Nafion/GOD/C–ZnO electrode in N_2- and air-saturated PBS solutions with and without the addition of 2 mM glucose. Scan rate: $0.5\,Vs^{-1}$. (c) Current–time curve of the Nafion/GOD/C–ZnO electrode for successive additions of glucose to air-saturated and stirred PBS solution at −0.45 V. (d) Amperometric response of the Nafion/HRP/C–ZnO electrode to H_2O_2 in PBS solution at −0.4 V. Inset is the calibration curve.

in Fig. 2b show that the electron transfer resistance at C–ZnO nanowire array electrode for $Fe(CN)_6]^{3-/4-}$ is ~85 Ω, much smaller than that at the pristine nanowire array electrode (~400 Ω), indicating its much improved electroactivity. The high electrocatalytic activity is most likely ascribed to both good conductivity and large specific surface area (C–ZnO: 55.4 $m^2\,g^{-1}$; Pristine ZnO:10.4 $m^2\,g^{-1}$) from the carbon deposited on ZnO. At Nafion/GOD/C–ZnO nanowire array electrode, the redox peak current increases as the scan rate increases and is linearly proportional to the scan rate ranging from 0.05 to $1\,Vs^{-1}$ (Fig. 3a), indicating a surface-controlled electrochemical process [17]. The electron transfer rate constant k_{ET} is calculated to be about $4.7 \pm 1.2\ s^{-1}$ using $k_{ET} = mnFv/RT$ [22],

which is much larger than those of GOD immobilized on SWCNT/MWCNT, TiO_2 and sol–gel silica modified electrodes [20,22,25]. It is believed that after the electron transfer from GOD to carbon, 1D single-crystal ZnO structures provide direct electron pathways for transferring electrons immediately from carbon to the electrode substrate.

In air-saturated PBS, dissolved oxygen could participate in the redox process [20], and a glucose biosensor can be readily developed based on the decrease of the reduction current upon the addition of glucose [22], as shown in Fig. 3b. Fig. 3c illustrates the amperometric response of the biosensor to successive additions of glucose at −0.45 V. The subsequent successive addition of glucose gives a continuous decrease of the current, with a fast average response time of ~5 s. The calibration plot is shown in the inset of Fig. 3c (A). It reveals a linear response range for glucose from 0.01 to 1.6 mM ($r = 0.999$, $n = 15$). From the slope the sensitivity of our biosensor is determined to be as high as 35.3 $\mu A\, mM^{-1}\, cm^{-2}$, better than many previous reports [14,15,20] and even higher than that of the biosensor constructed with Pt/CNTs(30 $\mu A\, mM^{-1}\, cm^{-2}$) [26]. The detection limit is further estimated as 1 μM at a signal-to-noise ratio(S/N) of 3. According to the Lineweaver-Burk equation [6], the apparent Michaelis–Menten constant K_M, an indicator of enzyme-substrate reaction kinetics, is calculated to be ~1.54 mM, indicating a high affinity of GOD to glucose on C–ZnO [4,10].

The low detection potential (−0.45 V) could eliminate the interference of other substrates such as ascorbic acid (AA) and dopamine (DA). The interference was investigated by using the relevant physiological levels (~0.1 mM) of these common interfering species, showing negligible responses (inset of Fig. 3c, B). When the biosensor was stored at 4 °C and measured at intervals of 5 days, it retained about 95% of its original sensitivity after one month. The fabrication reproducibility of five electrodes, made independently, showed reproducibility with the cumulative variation (CV) of 2.3% for the detection of glucose.

As another example, the detection of H_2O_2 based on the direct electron transfer of HRP [22] was also performed using the array electrode. Fig. 3d displays the amperometric response of Nafion/HRP/C–ZnO electrode upon the addition of H_2O_2 solution. The designed H_2O_2 biosensor also exhibits very good electrochemical performance with high sensitivity (237.8 $\mu A\, mM^{-1}\, cm^{-2}$) and fast response (4 s). The detection limit is 0.2 μM (S/N = 3), lower than previous results [19,21].

4. Conclusions

In summary, the fast direct electrochemistry of GOD was realized at a C–ZnO nanowire array electrode (Ti foil). An novel amperometric glucose biosensor based on the direct electrochemistry of GOD was constructed and showed high sensitivity and low detection limit. The H_2O_2 biosensor based on direct electrochemistry of HRP was also fabricated using C–ZnO array and also demonstrated good performance. With the combination of the merits of carbon and ZnO nanowire array on a conductive substrate, C–ZnO nanowire array represents an excellent platform for direct electrochemistry of proteins and biosensors.

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