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Short communication

Direct electrochemistry of glucose oxidase and glucose biosensing on a hydroxyl fullerenes modified glassy carbon electrode

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ABSTRACT

Direct electrochemistry of glucose oxidase (GOD) was achieved when GOD-hydroxyl fullerenes (HFs) nano-complex was immobilized on a glassy carbon (GC) electrode and protected with a chitosan (Chit) membrane. The ultraviolet–visible absorption spectrometry (UV–vis), transmission electron microscopy (TEM), and circular dichroism spectropolarimeter (CD) methods were utilized for additional characterization of the GOD, GOD-HFs and Chit/GOD-HFs. Chit/HFs may preserve the secondary structure and catalytic properties of GOD. The cyclic voltammograms (CVs) of the modified GC electrode showed a pair of well-defined quasi-reversible redox peaks with the formal potential ($E^{\circ\prime}$) of 353 ± 2 mV versus Ag/AgCl at a scan rate of 0.05 V/s. The heterogeneous electron transfer constant (k_s) was calculated to be $2.7 \pm 0.2\ s^{-1}$. The modified electrode response to glucose was linear in the concentrations ranging from 0.05 to 1.0 mM, with a detection limit of 5 ± 1 μM. The apparent Michaelis–Menten constant (K_m^{app}) was 694 ± 8 μM. Thus, the modified electrode could be applied as a third generation biosensor for glucose with high sensitivity, selectivity and low detection limit.

1. Introduction

Direct electrochemistry between a redox protein and electrode provides a suitable model for understanding the electron transfer mechanisms in biological systems. It also helps to establish a platform for fabrication of electrochemical devices such as third generation biosensors (Armstrong and Wilson, 2000; Hill, 1996; Thevenot et al., 2001).

Glucose oxidase (GOD) is a structurally rigid glycoprotein with a molecular weight of about 150–180 kDa. It catalyzes the electron transfer from glucose to oxygen accompanying the production of gluconic acid and hydrogen peroxide (Hecht et al., 1993). Direct electrochemistry of GOD has been realized and studied when it was immobilized on various types of modified electrodes, (Cai and Chen, 2004; Cui et al., 2011; Dai et al., 2009; Deng et al., 2008; Fu et al., 2009; Gu et al., 2011; Liu et al., 2007; Periasamy et al., 2011; Qiu et al., 2012; Tasviri et al., 2011; Wang et al., 2009; Yang et al., 2008; Zhai et al., 2011; Zhang et al., 2004; Zhang et al., 2011). However, these modifications may alter the conformation or structure of GOD (Calzolai et al., 2010; Shang et al., 2007), and result in poor selectivity and limited practical use of the modified electrodes.

Hydroxyl fullerenes (HFs) are water soluble fullerene derivatives with a formula of $C_{60}(OH)_n$. The number of –OH groups (n) is typically from 18 to 24. HFs are useful for the formation of gels, starburst polymers, and composites, and may be explored for biological activity (Chiang et al., 1996). HFs may link with a protein and form specific noncovalent complexes, and could be used to protect proteins (She et al., 1998).

In the present report, a novel GOD-HFs nano-complex was self-assembled and immobilized on a glassy carbon (GC) electrode. The electrode was then covered with a chitosan (Chit) membrane for protection and used to measure the direct electron transfer of

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GOD. Our results showed that the designed electrode can be used as a biosensor for detection of glucose with high sensitivity and selectivity.

2. Materials and methods

2.1. Materials

GOD (EC 1.1.3.4, from *aspergillus niger*), horseradish peroxidase (HRP, EC 1.11.1.7), guaiacol, Chit (0.5% in water), α-D-glucose, ascorbic acid (AA), uric acid (UA) and dopamine (DA) were from Sigma (Saint Louis, MO, USA). HFs was purchased from Bucky (Houston, USA). All solutions were prepared in double-distilled deionized water. All other chemicals were of analytical grade and used without further purification.

2.2. Preparation of Chit/GOD-HFs modified GC electrode

The procedure for the preparation of the GC electrode was as previously described (Hong et al., 2013). Prior to coating, the GC electrode was mechanically polished twice with alumina. The electrode was then treated electrochemically in 0.2 M sulfuric acid. Thereafter, the working electrode was placed in a 50 mM PBS (pH 7.0), and an anodic potential of 1.70 V (versus Ag/AgCl) was applied for 3–5 min. After the electrode was washed, 3 μl of the mixture of HFs (2 mg/ml) and GOD (10 mg/ml) (ratio of volume: 1) was dropped onto the surface of the electrode, and dried for 24 h at 4 °C, and for protection, 2 μl 0.5% Chit solution was dropped on the electrode surface, dried and stored at 4 °C.

2.3. Apparatus and measurements

All electrochemical experiments were carried out on a CHI650C (CHI Instrument, Austin, USA). An Ag/AgCl-saturated KCl, a Pt wire, and a GC electrode of 3 mm diameter (CHI Instrument, USA) were used as reference, counter, and working electrodes, respectively. The electrochemical measurements were carried out in N_2-saturated 50 mM sodium phosphate buffer solution (PBS, pH 7.0) at 25 ± 1 °C. The electro catalytic measurements were carried out after air bubbling (30 min, 300 ml/min).

After being mixed and incubated for half an hour, the far-UV spectra of GOD, GOD-HFs or Chit/GOD-HFs were performed on an Aviv Model 420SF circular dichroism spectropolarimeter (CD) (Lakewood, NJ, USA) at 25 °C, respectively, using rectangular quartz cells with a path length of 1.0 mm for far-UV (Farivar et al., 2010; Hong et al., 2012).

The catalytic rates of GOD, GOD-HFs and Chit/GOD-HFs were obtained, respectively, on a TU-1901 spectrophotometer (Beijing Purkinje General Instrument Company, China), using a 1 cm path-length cell equipped with a thermostat holder and an external temperature controller (Shanghai Hengping Instrument Company, China) at 25 ± 0.1 °C, based on two step reactions: Step 1, GOD catalyzes glucose to produce H_2O_2 in the presence of O_2. Step 2, the guaiacol is oxidized by H_2O_2 produced in Step 1 in the presence of HRP and the reaction rate was monitored at 470 nm. The colored product from the Step 2 is tetraguaiacol with an extinction coefficient of 26.6 $mM^{-1}\,cm^{-1}$ (at 470 nm), and the initial rate of guaiacol oxidation was measured by the rate of tetraguaiacol formation (Koduri and Tien, 1995).

Electron macrograph images of Chit/GOD-HFs sample were obtained using a TEM (JEM-1400, JEOL, Japan) operating at 80 kV.

3. Results and discussion

3.1. Characteristics of GOD, GOD-HFs and Chit/GOD-HFs

Fig. 1A and B shows the possible structure of GOD-HFs and TEM image of Chit/GOD-HFs, respectively. The TEM image (Fig. 1B) shows that GOD-HFs nano-particles may be formed in Chit membrane, with an average size of 20 nm. Fig. 1C is a schematic representation of the Chit/GOD-HFs modified GC electrode.

Fig. S1A shows the Far-UV-CD spectra of GOD, GOD-HFs and Chit/GOD-HFs in 50 mM PBS at pH 7.0. Fig. S1A inset table shows the detailed percentages of several secondary components of the GOD, GOD-HFs and Chit/GOD-HFs, which were obtained using Aviv software (CD spectra deconvolution). The percentage of helix decreased from 20% in GOD to 15.2% in GOD-HFs. However, after being modified with Chit, the percentage of GOD helix in Chit/GOD-HFs increased to 20.8%. Thus, the Chit membrane may help to preserve the GOD helix structure. The percentage of anti-parallel structure increased from 35.6% in GOD to 40.8% in GOD-HFs, and supports the possible structure schemed in Fig. 1A.

The catalytic rates of GOD, GOD-HFs, and Chit/GOD-HFs were compared through the initial reaction rate of guaiacol oxidation (Fig. S1B). The native GOD had the fastest initial reaction rate, while the GOD-HFs had the slowest reaction rate. This was mainly attributed to the conformational changes in GOD when combined with HFs. However, the initial reaction rate was improved for Chit/GOD-HFs due to excellent biocompatibility of Chit membrane. The specific noncovalent linking (hydrogen bond, van der vals or hydrophobicity interactions) between GOD and HFs in Chit membrane can occur (She et al., 1998). This may help to preserve the structure and function of GOD, and lead to higher sensitivity and selectivity of GOD-HFs modified GC electrode for glucose.

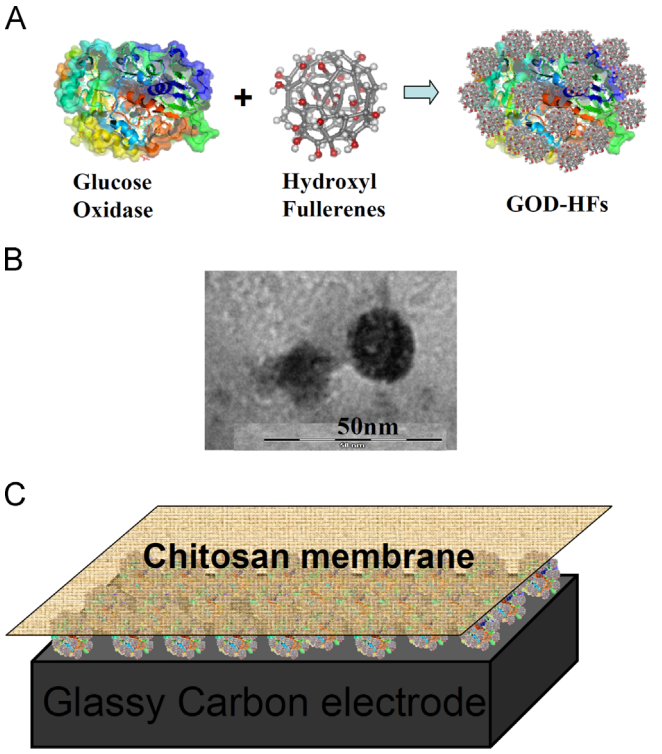

Fig. 1. (A) Possible structure of GOD-HFs, (B) transmission electron microscopy (TEM) image of Chit/GOD-HFs and (C) schematic structure of the Chit/GOD-HFs modified glassy carbon (GC) electrode.