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Direct Electrochemistry of Glucose Oxidase Immobilized on a Hydroxyl Fullerenes- TiO_2 Nanocomposite Modified Glassy Carbon Electrode

Di LI[1], Xiao-yu ZHANG[1], Bao-lin XIAO[1], Jun HONG[1,2,a,*] and Ali Akbar Moosavi-Movahedi [3,b]

[1]School of Life Sciences, Henan University, Kaifeng 475000, China

[2]Institute of Biotechnology, Henan University, Kaifeng 475000, China

[3]Institute of Biochemistry and Biophysics, University of Tehran, Tehran, Iran

[a]hongjun@henu.edu.cn

[b]moosavi@ut.ac.ir

*Corresponding author

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Abstract. By immobilizing glucose oxidase (GOD) on a nanocomposite containing hydroxyl fullerenes ($C_{60}-OH$) and TiO_2 nanoparticles, a new type biosensor was prepared for glucose detection. The cyclic voltammograms (CVs) of GOD on the nanocomposite modified glassy carbon electrode (GCE) was quasi-reversible. The heterogeneous electron transfer constant (ks) and the formal potential ($E^{o'}$) of the Chi/GOD/ $C_{60}-OH-TiO_2$ /GCE were 3.0 s^{-1} and -0.248V, respectively. The Chi/GOD/$C_{60}-OH-TiO_2$/GCE responsed linearly to glucose from 0.05 to 0.85 mM. The apparent Michaelis–Menten constant (K_m^{app}) and detection limit of the Chi/GOD/$C_{60}-OH-TiO_2$/GCE were 1.17 mM and 0.05 mM, respectively.

Introduction

Glucose oxidase (GOD) is a glycoprotein with a relative rigid structure. Its molecular weight is around 150–180 kDa. In the present of GOD, glucose reacts with oxygen to produce gluconic acid and hydrogen peroxide [1]. It is usually difficult to observe the direct electron exchange between GOD and electrode surface, because the FAD groups are buried deeply inside the polypeptide chains of relatively large GOD molecules. Direct electron transfer of GOD on various types of modified electrodes has been investigated. And nanoparticles, such as Ag, Au, MnO_2, Fe_3O_4 or TiO_2, and carbon nanotubes (CNTs) have also been served in electrochemical study of redox proteins [2-12]. Among them, the combination of several kinds of nano-materials may bring together their unique properties and generate a new nanocomposite with superior characteristics [13-15].

In this report, we presented a novel GOD-Hydroxyl fullerenes-TiO_2 nano composite immobilized glassy carbon electrode (GCE). The electrode was also covered with a layer of chitosan (Chi) film to improve its stability. The hydroxyl fullerenes (C_{60}-OH) may form specific noncovalent complexes with a protein and help to protect the protein [16-17].

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Experimental

Reagents

GOD (from aspergillus niger) and β-D-glucose were purchased from Sigma of USA. C_{60}-OH and TiO_2 nano particles were bought from Bucky (Houston, USA).

Preparation of Chit/GOD/C_{60}-OH—TiO_2/GCE

The procedure for the GCE preparation was as literature [18]. 2 μl of the mixture of TiO_2 nano particles and C_{60}-OH (2 mg/ml) (ratio of volume: 1) was dropped onto the surface of the GCE, and dry at room temperature for 20min. Then 2μl GOD (5 mg/ml) and 2 μl 0.5% Chi solution was added respectively, in the same steps. The schematic fabrication process of the Chit/GOD/C_{60}-OH—TiO_2/GCE was shown as Fig.1.

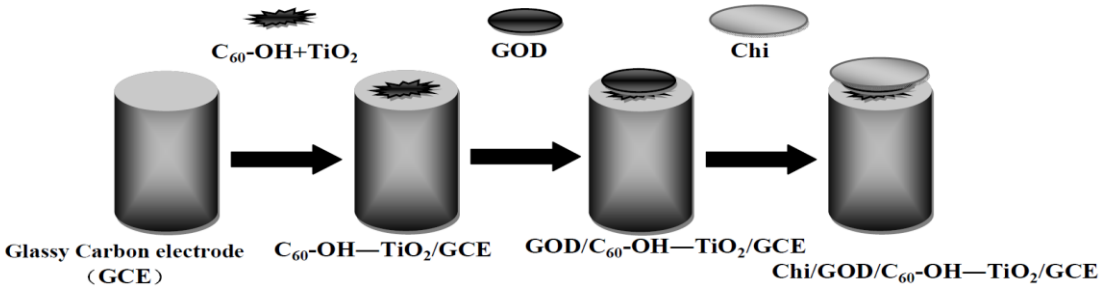

Fig.1 The schematic fabrication process of Chit/GOD/C_{60}-OH—TiO_2/GCE

Apparatus and Measurements

A CHI650C (CHI Instrument, Austin, USA) was applied for all electrochemical experiments. A GCE, an Ag/AgCl-saturated KCl and a Pt wire were served for electrochemical detections in 50 mM, pH 6.0 sodium phosphate buffer solution at 20 ± 1 ℃.

Electron macrograph images (TEM) of C_{60}-OH, TiO_2 nanoparticles, Chi/C_{60}-OH—TiO_2 and Chi/GOD/C_{60}-OH—TiO_2 were achieved using a TEM instrument (JEM-2100, JEOL, Japan) operating at 200kV.

Results and Discussion

Characteristics of Nano-materials

The TEM images of (A)C_{60}-OH, (B)TiO_2 nanoparticles, (C)Chi/C_{60}-OH—TiO_2 and (D)Chi/GOD/C_{60}-OH—TiO_2 were shown in Fig.2. It could be seen that the addition of Chit is helpful to the dispersion of nanomaterials into the membrane. In addition to catalysis, GOD also has a certain adhesive effect.

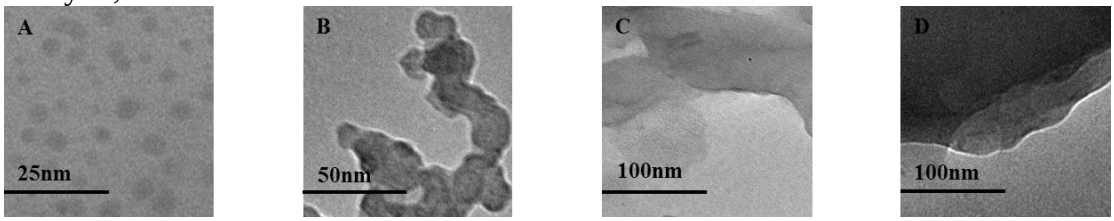

Fig.2 TEM images of (A)C_{60}-OH,(B)TiO_2 nanoparticles,(C)Chi/C_{60}-OH—TiO_2, (D)Chi/GOD/C_{60}-OH—TiO_2

Electrochemical Properties of Chi/GOD/C_{60}-OH—TiO_2/GCE

The cyclic voltammograms (CVs) of (a) Chi/GOD/C_{60}-OH—TiO_2/GCE (b) Chi/C_{60}-OH—TiO_2/GCE, and (c) bare GCE were shown in Fig.3. It could be seen that curve (a) shows a pair of stronger redox peaks respect to the others. The anodic and cathodic peak currents (Ipa and Ipc) of the Chi/GOD/C_{60}-OH—TiO_2/GCE were obtained to be 1.557 and 1.440 μA, respectively. The anodic and cathodic peak potentials (Epa and Epc) of the Chi/GOD/C_{60}-OH—TiO_2/GCE were obtained to be -0.231 and -0.265 V, respectively. Then the ration of Ipa/ Ipc (1.08) was close to 1. The ΔEp (Epa-Epc) was 0.034V. So, the electrochemical process of the Chi/GOD/C_{60}-OH—TiO_2/GCE (curve a) was a quasi-reversible process [19]. Moreover, the formal potential (E^0=Epa/2+Epc/2) of the Chi/GOD/C_{60}-OH—TiO_2/GCE was obtained to be -0.248V versus Ag/AgCl, which shows a +105 mV shift relative to that of Chi/GOD/C_{60}-OH/GCE (-0.353V)[15], this positive shift may facilitate the electrode reaction[19].

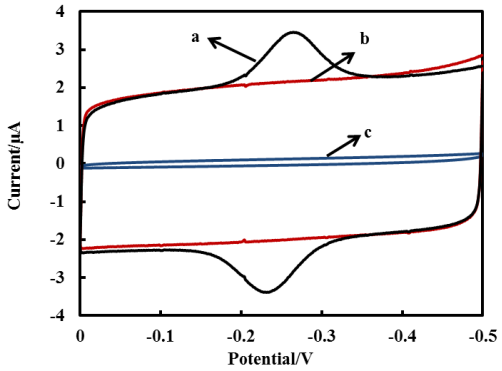

Fig.3 CVs of different modified electrodes: (a) Chi/GOD/C_{60}-OH—TiO_2/GCE (b) Chi/C_{60}-OH—TiO_2/GCE, and (c) bare GCE.

Effects of pH Value on the Chi/GOD/C_{60}-OH—TiO_2/GCE

The CVs of Chi/GOD/C_{60}-OH—TiO_2/GCE at different pH values were shown in Fig.4A. The formal potential of the electrode decreased linearly with the increase of pH value (Fig. 4B). The slope value (58.3 mV/pH) was similar to the theoretical value (59.2 mV/pH) [18]. The pH value of 6.0 was chosen as the optimal pH value for the next set of experiment because the maximum cathodic and anodic currents were achieved at pH 6.0.

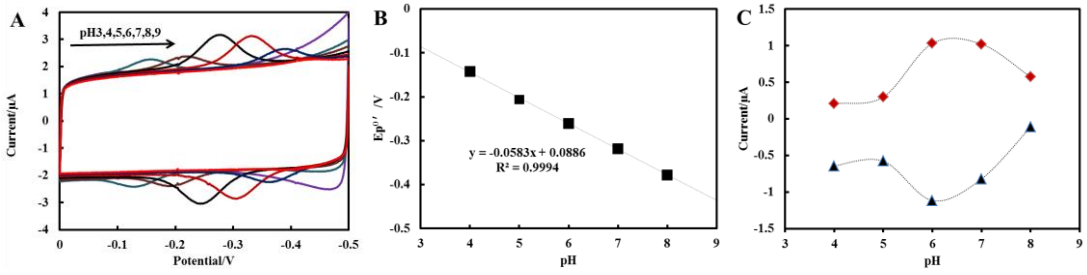

Fig. 4 CVs of Chi/GOD/C_{60}-OH—TiO_2/GCE at different pH values (from left to right): 3.0, 4.0, 5.0, 6.0, 7.0, 8.0 and 9.0, respectively, at a scan rate of 0.05V/s.

Electrochemical Properties of the Chi/GOD/C_{60}-OH—TiO_2/GCE

The effects scan rates in the CVs investigation on the Chi/GOD/C_{60}-OH—TiO_2/GCE were shown in Fig.5A. The peak current value of the Chi/GOD/C_{60}-OH—TiO_2/GCE increased linearly with the increase of scan rate (ν) (0.01-0.3V/s (Fig. 5B), and the Chi/GOD/C_{60}-OH—TiO_2 was stably immobilized on the GCE [19]. The cathodic peak potential (Epc) of the Chi/GOD/C_{60}-OH—TiO_2/GCE changed linearly versus lnυ (where, υ was from 0.5 to 1.0V/s) (Fig. 5C). The α and n were 2 and 0.39, respectively [20-21]. The heterogeneous electron transfer rate constant (ks) of the Chi/GOD/C_{60}-OH—TiO_2/GCE was obtained to be 3.0s-1 [22].

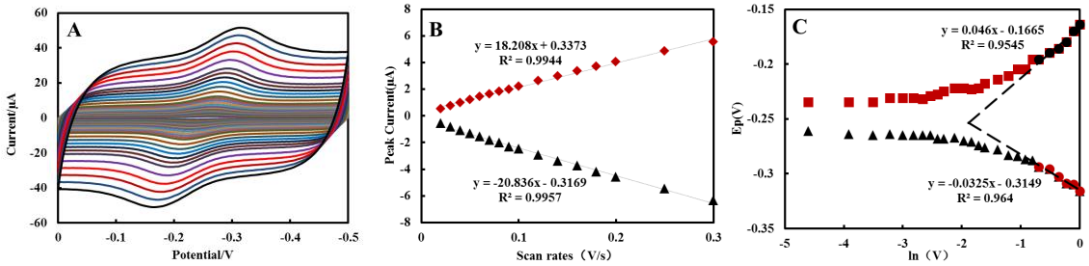

Fig.5 (A) The CVs of Chi/GOD/C_{60}-OH—TiO_2/GCE at various scan rate. (B) Plot of Ip versus υ. (C) Plot of Ep versus lnυ.

Electro-catalytical Properties of the Chi/GOD/C_{60}-OH—TiO_2/GCE

The linear sweep voltammograms (LSVs) and the amperometric response of Chi/GOD/C_{60}-OH—TiO_2/GCE in different concentrations of glucose (0-5mM) were shown in Fig. 6A and 6B, respectively. The linear detection range of the Chi/GOD/C_{60}-OH—TiO_2/GCE was obtained to be from 0.05 to 0.85 mM. Furthermore, the detection limit (Fig. 6C) and the apparent Michaelis–Menten constant (K_{mapp}) (Fig.6D) of the Chi/GOD/C_{60}-OH—TiO_2/GCE were obtained to be 0.05 mM and 1.17 mM, respectively.

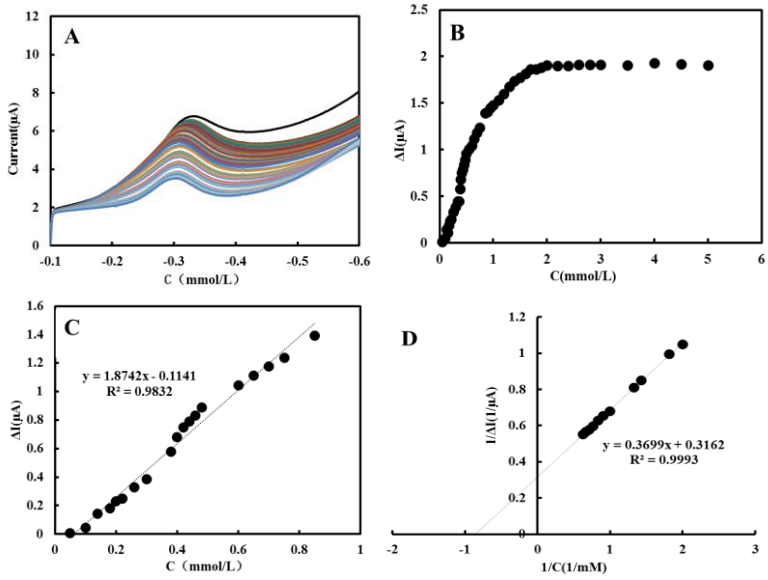

Fig.6 (A) LSVs of Chi/GOD/C_{60}-OH—TiO_2/GCE in the presence of different concentrations of glucose (0-0.85mM). (B) Amperometric response of Chi/GOD/C_{60}-OH—TiO_2/GCE toward glucose. (C) The linear detection range and (D) Lineweaver–Burk plot of the Chi/GOD/C_{60}-OH—TiO_2/GCE.

Conclusions

Direct electron transfer of GOD was realized and investigated when GOD was immobilized on a C_{60}-OH and TiO_2 nanocomposite modified GCE. The Chi/GOD/C_{60}-OH—TiO_2/GCE could be applied as a sensitive biosensor for glucose.

Acknowledgment

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