Glucose Biosensing Using Glassy Carbon Electrode Modified with Polyhydroxy-C60, Glucose Oxidase and Ionic-liquid

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Abstract. Direct electrochemistry of glucose oxidase (GOD) was achieved when an ionic liquid/GOD-Polyhydroxy-C60 functional membrane was confined on a glassy carbon electrode (GCE). The cyclic voltammograms (CVs) of the modified GCE showed a pair of redox peaks with a formal potential (E\(^{\circ}\)) of -329±2 mV. The heterogeneous electron transfer constant (k\(_s\)) was 1.43 s\(^{-1}\). The modified GCE response to glucose was linear in the range from 0.02 to 2.0 mM. The detection limit was 1 µM. The apparent Michaelis-Menten constant (K\(_{m,app}\)) was 1.45 mM.

Keywords: Glucose oxidase, polyhydroxy-C60, ionic liquid, direct electrochemistry

1. Introduction

Fullerenes are outstanding stable clusters, consisting of sp2-hybridized carbon atoms arranged in a 3D structure with a sphere-like shape [1–3] and characterized by hydrophobicity, high thermal stability and stability to oxidation [4]. Functionalized fullerene derivatives have been used in the areas of nanotechnology and materials science because of their unique photophysical and electrochemical properties [5]. Polyhydroxy-C60 (PHC) is a water soluble functionalized fullerene derivatives [6,7]. PHC could link with a protein and form specific complex, and could be used to protect proteins [8].

Ion liquids (IL) can form organized polymeric supramolecules containing polar and non-polar regions in either liquid or solid states [9,10]. In solution, proteins (or enzymes) could be embedded in IL network, and the native structure of proteins could be preserved [10–12].

In this work, a functional membrane was improved by using IL (1-Butyl-3-methyl imidazolium tetrafluoroborate, [BMIM] BF\(_4\)), Glucose oxidase (GOD) and PHC. Noncovalent linking (hydrogen bond, van der vals or hydrophobicity interactions) between GOD and PHC in IL membrane have been formed, which may help to preserve the stability of GOD’s structure and function. Direct electron electrochemistry of GOD was achieved when the functional membrane was confined on a glassy carbon electrode.
carbon electrode (GCE). The modified electrode could be used as a new type biosensor for detection of glucose with good sensitivity, stability and selectivity.

2. Experimental

2.1. Reagents and apparatus

GOD (from *Aspergillus niger*), 1-Butyl-3-methyl imidazolium tetrafluoroborate (IL) and α-D-glucose were purchased from Sigma (Saint Louis, MO, USA). PHC was obtained from Bucky (Houston, USA). Hydrogen peroxide was from Shanghai Chemicals Company (Shanghai, China). All other chemicals were of analytical grade and used without further purification. All solutions were prepared in double-distilled deionized water.

All electrochemical experiments were carried out in a three electrode cell electrochemical system (CHI650C, CHI Instrument, Austin, USA). A Pt wire, an Ag/AgCl, and a GCE (3mm diameter) were served as the counter, reference and working electrodes, respectively. The electrochemical detections were done in N₂-saturated 50mM, pH 7.0 phosphate buffer solution (PBS) at room temperature.

2.2. Fabrication of IL/GOD/PHC functional membrane modified GCE

The procedure for the preparation of the GCE was as following [13–19]: At first, the GCE was polished twice with alumina (1.00, 0.30 and 0.05 μm, respectively). Then the GCE was treated in 0.2 M H₂SO₄. Thereafter, the electrode was dipped in PBS at 1.70 V for 3-5 min, washed with water. Then, 3μl of the mixture of PHC (2 mg/ml) and GOD (10 mg/ml) (ratio of volume: 1) was coated onto the surface of the electrode, and dried for 24 h in a refrigerator, 2 μl IL was dropped on the surface of the electrode, dried and kept in the refrigerator. The electro catalytic detections were done after air bubbling of 5 ml PBS and addition of 20 μl different concentration of glucose solution (or real blood sample).

3. Results and discussion

Figure 1 shows the preparation process of IL/GOD/PHC modified GCE. TEM image also shows that GOD-PHC nano particles may be formed with an average diameter of 20 nm (data not shown).
Figure 2A shows the cyclic voltammograms of bare GCE (a), IL/GOD/GCE (b), IL/PHC/GCE (c) and IL/GOD/PHC/GCE (d). A pair of redox peaks was observed at the IL/GOD/PHC modified GCE. The formal potential ($E^{\circ}$) was -329 ± 2 mV. This value was greater than the most reported results, for example, the $E^{\circ}$ obtained at GOD/Au/GCE (-444 mV vs SCE) [16], NF/GOD/CNT/GCE (-464 mV vs SCE) [17] and GOD/PDDA-HCNTs/MGC electrode (-471 mV vs Ag/AgCl) [18]. Positive potential shift for the electrode may lead to a more efficient electro catalytic reaction [14]. However, no redox peak was observed for the bare GCE (curve a), IL/GOD/GCE (curve b) and IL/PHC/GCE (curve c).

Figure 2(B) shows the cyclic voltammograms (CVs) of IL/GOD/PHC/GCE in 50 mM PBS (pH 7.0) at various scan rates. The peak currents increased linearly with increasing scan rate ($v$) (Figure 2C). The cathodic peak potential ($E_{pc}$) changed linearly versus $\ln(v)$ from 0.25 to 0.5 V/s (Figure 2D). Then, it could be concluded that $n = 2$ and $\alpha = 0.72$ [20] based on Eq. (1) [21]:

\[ y = 5.48x + 1.03 \]
\[ R^2 = 0.9986 \]

\[ y = -0.0356x - 0.4296 \]
\[ R^2 = 0.99902 \]
where R is the gas constant, T is the temperature (293 K here), $\alpha$ the cathodic electron transfer coefficient, $n$ the number of electrons, and F the Faraday constant, respectively.

The apparent heterogeneous electron transfer rate constant ($k_s$) was calculated to be $1.43 \times 10^{-1}$ s$^{-1}$, using the following equation based on Eq. (2) [22]:

$$\ln k_s = a \ln(1 - a) + (1 - a) \ln a - \ln\left(\frac{RT}{nF}\right) - a(1 - a) \frac{nF\Delta E_p}{RT}$$

The concentration ($\Gamma$) of GOD on the GCE was $5.33 \times 10^{-10}$ mol·cm$^{-2}$ based on Eq. (3) [23]:

$$I_p = \frac{n^2F^2A\Gamma v}{4RT}$$

This value of $\Gamma$ is larger than the theoretical $\Gamma$ value of $1.7 \times 10^{-10}$ mol cm$^{-2}$ based on PDB data of GOD (PDB ID: 1cF3, about 10 nm diameter) and the $\Gamma$ value $1.0 \times 10^{-11}$ mol cm$^{-2}$ based on the TEM image (about 20 nm diameter, data not shown).

CVs of IL/GOD/PHC/GCE at different pH values are shown in Figure 3A. The formal potential ($E^\circ$) of the modified GCE was pH dependent with a slope of 51.7 mV/pH (Figure 3B). This value was close to the Nernst’s value of 59.2 mV [15]. From Figure 3C, it could be seen that the optimized potential for amperometric detection of glucose was -0.35V versus Ag/AgCl [15,19]. The modified electrode linearly responded to glucose at concentration ranging from 0.02-2.0 mM. The detection limit was 1μM. This detection limit value was smaller than most reported results [24–28].
apparent Michaelis-Menten constant ($K_{m\text{app}}$) was calculated to be 1.45 mM from the electrochemical version of the Lineweaver-Burk Eq. (4) (data not shown) [19].

$$\frac{1}{I_s} = \frac{1}{I_{\text{max}}} + \frac{K_{m\text{app}}}{I_{\text{max}}c}$$  \hspace{1cm} (4)

Where, $I_s$, $I_{\text{max}}$, and $c$ are steady-state current after the addition of glucose, maximum current measured under saturated glucose conditions and glucose concentration, respectively. $K_{m\text{app}}$ value was lower than the modified electrodes of PEI/GOD/PGE (7.9 mM) [24], Chit/DNA/GOD/Chit/GC (3.12 mM) [25] and GOD/NH$_2$-TiO$_2$-CNT/GC [26], and larger than the modified electrodes of GOD/BCNT/GC electrode (0.2 mM) [27] and GOD-GQD/CC electrode (0.76 mM) [28]. The low $K_{m\text{app}}$ value indicates a high affinity of glucose for IL/GOD/PHC/GCE. PHC may reduce the electron transfer distance between FAD group of GOD and the GCE [19].

Electro active species such as ascorbic acid (0.5 mM), uric acid (0.5 mM) and dopamine (0.5 mM) could cause negligible effect on the glucose detection, and IL/GOD/PHC modified GCE has a good selectivity on the glucose in serum samples (data not shown). The stability of the IL/GOD/PHC/GCE was checked by examining the cyclic voltammetric peak currents of GOD by continuously scanning for about 30 cycles (data not shown). There was nearly no decrease of the voltammetric response, showing that the modified GCE was stable in PBS solution.

4. Conclusion

Direct electrochemistry of GOD was achieved when GOD/PHC nano structure was confined on a GCE and protected with IL membrane. The modified GCE could be used as a third generation biosensor for determination of glucose with good sensitivity, stability and selectivity.

Acknowledgement

The support of Development Projects of Henan Province Science and Technology (No.142300410124) and Henan University Science Foundation is gratefully acknowledged.

References

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