Development of a Glucose Biosensor Using Advanced Electrode Modified by Nanohybrid Composing Chemically Modified Graphene and Ionic Liquid

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Abstract
Nanohybrids of chemically modified graphene (CMG) and ionic liquid (IL) were prepared by sonication to modify the electrode. The modified CMG-IL electrodes showed a higher current and smaller peak-to-peak potential separation than a bare electrode due to the promoted electron transfer rate. Furthermore, the glucose oxidase (GOx) immobilized on the modified electrode displayed direct electron transfer rate and symmetrical redox potentials with a linear relationship at different scan rates. The fabricated GOx/CMG-IL electrodes were developed selective glucose biosensor with respect to a sensitivity of 0.64 μA mM⁻¹, detection limit of 0.376 mM, and response time of < 5 s.

Keywords: Chemically modified electrode, Ionic liquids, Electron transfer, Glucose biosensor, Electrochemistry, Biosensors
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1. Introduction

Recently, the excellent electrochemical properties of composite electrodes based on the carbon materials have been unveiled and their applications toward electrochemical sensors and biosensors have been of particular interest in material science, biotechnology, human safety, and many other fields [1–5]. Of the carbon-based nanofillers, carbon nanotubes (CNTs) make them highly attractive as advanced engineering composite electrodes with various binders such as, Nafion [1], ionic liquids (ILs) [2], and Teflon [3], due to their ability to promote the electron transfer reactions of important biomolecules, including glucose, organophosphate, or NADH. One of the most promising ways to prepare the composite electrode with using CNTs is to modify the electrode surface with the dispersed CNT in a suitable solvent because of the easy, fast, and versatile method, inducing a high sensitivity, fast response time, and low detection limit [4, 5]. The improved performance of the biosensors with using these types of composite electrodes depended on the properties of the nanomaterials with attractive electrochemical and mechanical properties and the binders with compatibility at the interface between biomolecules and electrodes.

Of the many important nanomaterials for sensor applications, the chemically modified graphene (CMG) derived from graphene oxide has more promising candidates for practical biosensor applications than other nanomaterials in terms of its high electrochemical properties, scalability, processability, and versatility [6–8]. In particular, Li et al. reported graphene-modified electrode for detection of dopamine with well-defined sensitivity [6]. The use of exfoliated graphite nanoplatelets coated with Nafion by Lee et al. showed that the nanocomposite electrodes provide the improved electron transfer reaction of glucose with a high sensitivity and fast response time [7]. In addition to graphene, ionic liquids (ILs) have been widely used as another important material in enhancing performance of the modified electrode as a binder for biosensor because of their electrochemical stability, low back ground current, and biocompatibility [5]. ILs in previous works showed that they are effective solubilizing agents for CNTs and graphene nanosheets via π–π interactions, exhibiting improved sensitive signal for the detection of various analytes such as organophosphate [2], glucose [4], and ascorbic acid [9].

Herein, we demonstrated that the fabrication of composite electrode assembly using nanohybrids of CMG-IL prepared by sonication was developed the dramatically electrochemical changes of glucose biosensor. The nano-
composites based on the CMG nanofiller in this research showed the ability to act as modifiers to the electrode in the direct electrochemistry of glucose oxidase, and was subsequently investigated by cyclic voltammetry (CV), AC impedance, and amperometry. The synthesis of nanohybrids and fabrication of the biosensors could provide a facile and effective approach to build the electrochemical devices using the CMGs.

2. Experimental

2.1. Materials

Graphite powder and hydrazine solution (35 wt% in water) were purchased from Sigma-Aldrich. Glucose oxidase (GOX) (EC 1.1.3.4, lyophilized powder, 100 – 250 units/mg from Aspergillus niger) and β-D(+) glucose (97%) were obtained from Sigma. The IL, 1-butyl-3-methylimidazolium hexafluorophosphate ([bmim][PF₆]), were supplied with high purity of 99.9% by C-TRI. The ammonia solution was obtained from Junsei (28 wt%). Aqueous solutions were prepared with double-distilled water.

2.2. Synthesis of CMG and CMG-IL Nano-hybrids

Graphite oxide (GO) was synthesized from graphite powder by a modified Hummers method [10]. CMG nanosheets were prepared by following procedures [8]. As-purified GO suspensions were then dispersed in water to create 0.05 wt% dispersion by sonication for 30 min. In order to reduce the exfoliated GO, the homogeneous dispersion (5 mL) was mixed with 5 mL of water, 5 μL of hydrazine solution, and 35 μL of ammonia solution, and then was put in a water bath at 95°C for 1 h. Finally, black CMG papers were obtained after washing and filtering with water several times, and then dried in a vacuum. As-prepared CMG papers with 0.1 wt% were mixed with 2 g of [bmim][PF₆]. The CMG-IL composites were produced by the stable black dispersion of CMG-[bmim][PF₆] solution obtained from sonication for 1 h.

2.3. Preparation of CMG-IL and GOx/CMG-IL Modified Electrode

Prior to the surface modification, the gold (Au) electrodes were pretreated by polishing with 0.05 μm alumina powders and then ultrasonically cleaned with deionized water, and dried at room temperature in a vacuum. The CMG-IL modified electrodes were fabricated by dropping 5 μL of CMG-IL nanocomposite solution onto the Au electrode surface and dried overnight naturally. Then the CMG-IL/Au electrode was soaked in 5 mg mL⁻¹ of GOX solution in phosphate-buffered saline (PBS, pH 7.4). The GOX/CMG-IL/Au electrodes were obtained after washing with PBS to remove loosely bound GOX molecules. A control electrode (CMG-oil) was also prepared similarly by using mineral oil instead of IL.

3. Results and Discussion

3.1. Characterization of CMG-IL Nano-hybrids

The development of biosensors using graphene sheets was faced with serious two obstacles that must be overcome: first, achieving complete exfoliation of graphite and second, stabilizing this exfoliated graphene in a suspension in order to immobilize the enzymes in a large surface area [11]. In this regard, creating colloidal suspensions of CMGs by the introduction of negatively charged carboxylate groups was suitable for preparation of biosensor due to the nonaggregation of graphene sheets [1]. In particular, the combination of CMGs and [bmim][PF₆] via π–π interaction could provide the stabilization of CMGs and good compatibility with enzymes. The morphologies of two-dimensional CMGs and CMG-IL hybrids were confirmed by TEM images in Figure 1A and 1B, exhibiting that ILs were successfully attached onto the CMG nanosheets. The thickness of CMGs used in this work was confirmed by AFM images (Figure 1C) with 2.5 nm of thickness.

FT-IR spectroscopy was performed to identify the chemical groups of GOs, CMGs, and CMG-IL hybrids. The spectrum of the GOs in Figure 1D shows the presence of O–H (νO-H at 3368 cm⁻¹), C=O (νC=O at 1722 cm⁻¹), C=C (νC=C at 1655 cm⁻¹), and C–O (νC-O at 1231 cm⁻¹ in epoxy groups, at 1065 cm⁻¹ in alkoxo groups) [8]. After reduction with hydrazine, the decrease of adsorption bands of oxygen functionalities, which provide evidence of CMG synthesized successfully from GOs [8, 12]. The FT-IR spectrum of the
CMG-IL nanosheets revealed \([\text{bmim}]\text{PF}_6\) absorption peaks, such as C–N (\(\nu_{\text{C–N}}\) at 1168 cm\(^{-1}\)), C=C (\(\nu_{\text{C–C}}\) at 1572 cm\(^{-1}\)), and PF\(_6^-(\) near 830 cm\(^{-1}\)) \([13, 14]\).

Figure 2 shows the SEM images of GOx/CMG-IL deposited on the Au electrode. At a low magnification, most of the basal planes of CMG coated with IL were deposited on the surface of Au electrode and some of the edge planes of CMG were curled or protrude from the surface. The globular GOx was uniformly absorbed on the CMG-IL sheets from the SEM images at a high magnification.

3.2. Electrochemical Properties of Modified Electrodes

To investigate the effect of CMG nanofiller on the electrochemical properties, the CVs were examined as shown in Figure 3A. After the Au electrodes were modified with CMG-IL, the peak current increased dramatically with a decrease of the peak-to-peak potential separation (\(\Delta E_p\)) from 74 mV for the bare Au to 68 mV for the CMG-IL/Au electrode. The CMG-IL/Au electrode had a higher peak current and smaller \(\Delta E_p\) than those of the IL/Au electrode, indicating the electron transfer promoting effect of CMG nanofiller. When GOx was adsorbed on the surface of the CMG-IL/Au electrode, the peak current decreased and \(\Delta E_p\) (72 mV) increased comparing with that of the CMG-IL/Au electrode, resulting from the electron transfer resistance of adsorbed GOx molecules.

The equivalent circuit of the composite electrodes described in Figure 3B shows clearly the separated contributions of the bulk and the interface to the overall resistance in composite electrodes by analyzing the AC impedance. The diameter of a semicircle on the \(Z’\) axis, which indicates the electron transfer resistance (\(R_{\text{CT}}\)) of the modified electrode, controls the electron transfer rate of redox agents at the electrode interface \([15]\). The charge transport resistances (\(R_{\text{CT}}\)) of CMG-IL/Au and CMG-oil/Au electrodes were estimated by using the software containing a CHI electrochemical workstation. \(R_{\text{CT}}\) of CMG-IL/Au electrode (143.67 k\(\Omega\)) in the Nyquist plot was lower than that of CMG-oil/Au electrode (8.71 k\(\Omega\)), indicating the binder role of IL in the surface-modified electrode due to the facilitated interfacial electron transfer between the composite electrode and electrolyte. These results indicate that the use of IL in the modified electrode with CMG provided an excellent binder between the electrode and electrolyte, inducing to the promotion of the electron transfer rate.
3.3. Direct Electrochemistry of GOx Immobilized on CMG-IL Modified Au Electrode

The understanding of direct electron transfer (DET) between the electrode and the redox enzymes such as GOx has been gained much attention in terms of academic point of view and practical construction of biosensors [16]. The direct electrochemistry of GOx based on redox-active centers, flavine adenine dinucleotide (FAD), was confirmed by the CV experiments in the potential range from −0.8 V to 0 V, following the previously proposed reaction mechanism [17].

\[
\text{GOx(FAD)} + 2\text{H}^+ + 2e^- \rightleftharpoons \text{GOx(FADH}_2
\]  

in the case of no oxygen

As shown in Figure 4A, no responses were observed on the bare Au surface in the presence of GOx and in the absence of GOx. After immobilization of GOx on the CMG-IL modified Au electrodes, the redox reaction of FAD/FADH\(_2\) was appeared in N\(_2\)-saturated PBS solution at a scan rate of 50 mV s\(^{-1}\).

Figure 4B presents the CV of GOx/CMG-IL/Au electrode at a series of scan rates ranging from 50 to 400 mV s\(^{-1}\). The inset shows that the symmetrical anodic and cathodic peaks have a linear relationship with a scan rate. The linear regression equations are \(I_{pa} (\mu A) = 5.46 + 4.34 \times 10^{-2} \nu (\text{mV s}^{-1})\), \(R^2 = 0.99796\), \(I_{pc} (\mu A) = -8.69 - 3.85 \times 10^{-2} \nu (\text{mV s}^{-1})\), \(R^2 = 0.99787\), respectively, revealing that the electron transfer reactions of the composite electrode were a surface-confined mechanism [18]. According to Laviron’s Equation 2 [19], electron transfer number was calculated to be 1.78, being consistent with the reported result [18].

\[
I_p = n^2F^2A^2 v (4RT)^{-1} = nFQv (4RT)^{-1}
\]  

The surface coverage (\(\Gamma^*\)) of GOx adsorbed on the CMG-IL/Au electrode was estimated to be \(1.66 \times 10^{-11} \text{ mol cm}^{-2}\), indicating an electroactive monolayer of GOx adsorbed on the surface of the CMG-IL/Au electrode. The DET of CMG-IL composite electrodes can be explained by the following
reasons related to the electronic structure, morphology and surface functional groups. Cai et al. reported that oxygen groups such as \(-\text{OH}, -\text{COOH}, \) and \(-\text{C}=\text{O}\) on the surface can promote the DET of GOx [1]. In addition, the edge defect sites of graphene had a high density of electronic states (DOS) at the Fermi level other than basal planes of graphene with a low DOS at the Fermi level [20]. In the case of two phase systems, when the Fermi levels of two phases were equal, a high DOS can promote fast electron transfer rate. Therefore, the curled edges of CMG-IL toward electrolyte with many carboxyl groups (FT-IR result; Fig.1D) and high DOS after immobilization of GOx were responsible for the direct electron transfer of GOx on the surface of composite electrodes.

3.4. Direct Electrocatalytic Analysis for the Glucose Detection

The direct bioelectrocatalytic activity of GOx immobilized on the CMG-IL/Au electrodes for oxidation of glucose was examined by CV under O\(_2\)-saturated PBS at different concentrations of glucose, as shown in Figure 5. The CVs of GOx/CMG-IL/Au electrodes represented a decrease of cathodic peak current following the increase of the glucose concentration due to the direct reduction of GOx with glucose at the electrode surface based on the following reaction [12, 17, 21].

\[
\text{GOx(FADH}_2\text{)} + \text{O}_2 \rightarrow \text{GOx(FAD)} + \text{H}_2\text{O}_2 \quad (3)
\]

\[
\text{GOx(FAD)} + \text{Glucose} \rightarrow \text{GOx(FADH}_2\text{)} + \text{Gluconolactone} \quad (4)
\]

The calibration curve corresponding to the decrease of the cathodic peak current at \(-0.513\) V is linear against the concentrations of glucose ranging from 1 to 6 mM \((R^2 = 0.9987)\) as shown in inset of Figure 5.

To demonstrate the performance of the DET glucose biosensor, the amperometric responses at the GOx/CMG-IL/Au glucose biosensor for each successive addition of 2 mM glucose at potential of \(-0.3\) V were also performed in order to demonstrate the performance of the DET glucose biosensor. Well-defined current responses for each concentration of glucose were obtained at biosensor as shown in Figure 6; the inset (a) represents the calibration curve. The response time, generating 95% of steady-state current signal, was very fast within 5 s. The linear response behavior at GOx/CMG-IL/Au electrode was obtained in the concentration range of glucose from 2 mM to 20 mM, which is enough for practical application in the detection of blood glucose [22, 23]. The calculated detection limit of glucose biosensor based on a signal-to-noise ratio of 3 was 0.376 mM. The well-fabricated GOx/CMG-IL/Au composite electrodes provided the direct electrochemistry due to the fast electron transfer rate, high surface area with many edge sites of CMG nanosheets, and easy accessibility to
immobilization sites of GOx attributed to the favorable morphology, and subsequently the linear glucose response behavior in this system was suitable for the clinical application to determine the blood sugar concentration as a model system for biosensor.

Selectivity of glucose biosensor is one of the most important factors for electrochemical biosensors due to the interferences of the common existing species with glucose such as ascorbic acid (AA) and uric acid (UA) for determination of glucose [23]. The selectivity of GOx/CMG-IL/Au electrode in the inset (b) of Figure 6 represents that the interference species do not affect the response of glucose at −0.3 V, but strong response was obtained at 0.4 V for glucose, AA, and UA. In order to investigate the stability of the GOx/CMG-IL/Au electrode, an operation of 100 cycles in a potential ranging from 0 to −0.8 V was performed, showing the absence of any significant change of the current response. The GOx/CMG-IL/Au electrode was stable in electrochemical response after immersion for 2 weeks in PBS at 4 °C.

4. Conclusions

Owing to the desirable electrochemical characteristics of the well-designed CMG-IL hybrids using a sonic system, the advanced modified electrodes were used for the development of electrochemical glucose biosensor. The easily fabricated glucose biosensor shows good electrochemical properties with fast response time (< 5 s), high selectivity, and well-defined linear response in the concentration range of glucose from 2 mM to 20 mM based on the direct electron transfer. This performance is achieved not only by using the distinct electrochemical properties of the CMG but also more importantly by optimizing the dispersion and interface chemistry assisted with IL to create the favorable interfacial area of the CMG contacted with electrolyte. Combining the versatile, facile and rapid attractive electrocatalytic behavior of CMG and IL paves the great potentials toward the development of advanced electrodes for various electrochemical sensors.

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References


Fig. 6. Amperometric responses of the GOx/CMG-IL/Au electrode with respect to 2 mM increments of glucose in O₂-saturated PBS solution at −0.3 V. Inset: (a) the calibration curves of the GOx/CMG-IL glucose biosensor at −0.3 V and (b) amperometric responses of the GOx/CMG-IL/Au electrode to adding 0.1 mM AA, 0.2 mM UA, and 2 mM glucose at different applied potentials.