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Direct electrochemistry and electrocatalysis of hemoglobin on three-dimensional graphene modified carbon ionic liquid electrode

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ABSTRACT

In this paper three-dimensional graphene (3D-GR) was directly formed on the surface of carbon ionic liquid electrode (CILE) by electrodeposition. By using 3D-GR/CILE as the substrate electrode, a new electrochemical biosensor was prepared by immobilization of hemoglobin (Hb) on the electrode surface with a chitosan film. Electrochemical investigation indicated that a pair of well-defined redox peaks appeared on cyclic voltammogram, indicating the realization of direct electron transfer of Hb with the underlying electrode. The result can be ascribed to the porous structure of 3D-GR with high conductivity and big surface area. Based on the electrochemical data, the electron transfer coefficient (α) and the apparent heterogeneous electron transfer rate constant (k_s) were calculated to be 0.426 and 1.864 s^{-1}, respectively. The modified electrode displayed good electrocatalytic activity to the reduction of trichloroacetic acid (TCA), and the catalytic reduction peak current had a good linear relationship to TCA concentration in the range from 0.4 to 26.0 mmol/L with the detection limit of 0.133 mmol/L (3σ). Therefore a new third-generation electrochemical Hb biosensor based on 3D-GR/CILE was constructed with good stability and reproducibility.

1. Introduction

Direct electrochemistry of redox proteins on the working electrode has been the research challenges in the field of bioelectrochemistry, which can offer the basis for the electron transfer of active centers and the structure of redox enzymes [1], and the fabrication of biosensors, bioreactors and biomedical devices [2]. However direct electron transfer is difficult to be realized on the normal working electrodes due to the deep burying of electroactive centers and the unfavorable orientation of proteins on the electrode surface. Therefore different kinds of chemically modified electrodes have been devised for the realization of electron transfer of redox proteins [3,4]. Recently nanomaterials with various morphology had been used for the realization of protein electrochemistry with well-defined electrochemical responses appeared [5,6]. Also these protein modified electrodes exhibit excellent bioelectrocatalytic activity to different substrates [7–10]. Among the redox proteins that used in protein electrochemistry, hemoglobin (Hb) is commonly selected as the ideal model for its well-documented structure and commercial availability with moderate cost. Direct electrochemistry of Hb has been realized with various mediators, promoters or nanomaterials [11–16].

Graphene (GR) is a kind of sp^2 hybridized two-dimensional carbon nanomaterial with specific characteristics [17]. Due to its larger surface area and excellent electronic conductivity, GR has been used widely in the fields of electrochemistry and electrochemical sensors [18,19]. However GR is tend to aggregate due to the *Van der waals* and π–π restacking, which limits its practical applications. Recently three-dimensional (3D) GR with high connected network has aroused great interests [20]. 3D-GR with porous structure exhibits extremely large surface area, highly conductive pathways and lower resistance with enhanced mobility of charge carries [21]. Therefore 3D-GR has many potential applications in the fields of electrochemistry and electroanalysis. Liu et al. applied a 3D-GR micropillar based electrochemical sensor for the detection of phenol [22]. Yuan et al. fabricated a bimetallic PdCu nanoparticle decorated 3D-GR hydrogel for non-enzymatic amperometric glucose sensor [23]. Li et al. applied a high-density 3D-GR macroscopic object for the high-capacity removal of heavy metal ions [24]. Wang et al. prepared a 3D-GR scaffold supported thin

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film silicon anode for lithium-ion batteries [25]. Different methods had been proposed for 3D-GR synthesis, including chemical vapor deposition (CVD), template and electrochemistry etc. [26]. Due to the presence of 3D structure with ideally interconnected porous structure, electrons can be transferred with high efficiency and the analyte can be fully contacted with the electrode surface, which is benefit for the electrochemical reaction. Therefore the 3D-GR related materials have great potential applications in the field of electrochemistry.

Trichloroacetic acid (TCA) is a metabolic byproduct of CCl_4 under reduced oxygen tension, which has been used widely as a pre-emergence herbicide, a peeling agent for undamaged skin and tattoos, or the precipitation of macromolecules such as proteins and DNA in biochemistry [27,28]. Also it could be found in drinking water as the result of chlorine disinfection and in the industrial wastewater [29]. Due to the electrocatalytic ability of heme proteins to the reduction of TCA, redox proteins modified electrodes have been devised for TCA sensing [30–32]. The presence of redox proteins on the electrode surface can decrease the reduction overpotential of TCA with the decrease of the activation energy, and the electrocatalytic current is proportional to TCA concentration in a certain range.

In this paper a 3D-GR modified electrode was prepared and used for the immobilization of Hb with a chitosan (CTS) film. CTS has been widely reported as an immobilization matrix with excellent film-forming ability for the preparation of biosensor and bioreactor [15]. IL modified carbon paste electrode (CPE) was used as the substrate electrode due to the specific electrochemical properties of IL such as high ionic conductivity and wide electrochemical windows [33,34]. By incorporating IL in the traditional CPE as the binder and the modifier, carbon ionic liquid electrode (CILE) had been elucidated the advantages including resistivity toward electrode fouling, high rates of electron transfer and the inherent catalytic activity [35,36]. By using CILE as the substrate electrode, 3D-GR was electrodeposited on its surface with increased surface area and higher electron conductivity, which was beneficial for the further immobilization of Hb. Direct electrochemistry of Hb was investigated on the modified electrode with a pair of well-defined redox peaks appeared, indicating the direct electron transfer was realized and accelerated. So the proposed electrode was a potential sensing platform for the further construction of third-generation electrochemical sensors.

2. Experimental

2.1. Apparatus and chemicals

Bovine hemoglobin (Hb, MW. 64500, Sinopharm Chemical Reagent Co., China), 1-hexylpyridinium hexafluorophosphate ($HPPF_6$, Lanzhou Greenchem. ILS. LICP. CAS., China), graphene oxide (GO, Taiyuan Tanmei Tech. Ltd. Co., China), lithium perchlorate ($LiClO_4$, Chengdu Kelong Chemical Reagent Co., China), graphite powder (average particle size 30 μm, Shanghai Colloid Chemical Co., China) and trichloroacetic acid (TCA, Tianjin Kemiou Chemical Co., China) were used as received. 0.1 mol/L phosphate buffer solutions were used as the supporting electrolyte, which were kept in a nitrogen atmosphere during the electrochemical measurements. All the other chemicals used were of analytical reagent grade and all aqueous solutions were prepared with double-distilled water.

Voltammetric measurements were executed on a CHI 1210A electrochemical workstation (Shanghai CH Instrument, China). Electrochemical impedance spectroscopy (EIS) was performed on a CHI 660D electrochemical workstation (Shanghai CH Instrument, China). A conventional three-electrode system was used with a CTS/Hb/3D-GR/CILE as working electrode, a platinum wire as auxiliary electrode and a saturated calomel electrode (SCE) as reference electrode. Scanning electron microscopy (SEM) was recorded on a JSM-7100F scanning electron microscope (Japan Electron Company, Japan).

2.2. Construction of CTS/Hb/3D-GR/CILE

Based on the reported procedure [37], $HPPF_6$ based CILE was fabricated by hand-mixing 0.80 g of $HPPF_6$ and 1.60 g of graphite powder in a mortar and ground carefully. A portion of resulting homogeneous paste was packed into a glass tube cavity ($\Phi = 4$ mm) and the electrical contact was established through a copper wire to the end of the paste. The surface of CILE was polished on a weighing paper just before use.

3D-GR modified CILE was prepared by electroreduction with a potentiostatic method based on the reference [38]. Briefly, a freshly prepared CILE was placed in the 3.0 mg/mL GO dispersion solution that containing 0.1 mol/L $LiClO_4$ with magnetic stirring and N_2 bubbling. By applying the potential of −1.30 V for 300 s, a stable electrochemical reduced 3D-GR film can be formed on the surface of CILE. The modified electrode was rinsed with ultrapure water and dried in nitrogen atmosphere for the further modification, which was denoted as 3D-GR/CILE.

Electrochemical biosensor was prepared by dropping 8.0 μL of 10.0 mg/mL Hb solution directly onto the surface of 3D-GR/CILE surface, and then the electrode was dried at room temperature. Finally, 5.0 μL of 1.0 mg/mL CTS (in 1.0% HAc) solution was spread onto the surface of Hb/3D-GR/CILE and dried to get the modified electrode, which was stored at 4 °C when not in use. Other modified electrodes such as CTS/CILE, CTS/3D-GR/CILE, CTS/Hb/CILE etc. were prepared with the similar procedure for comparison.

3. Results and discussion

3.1. Characteristics of the modified electrodes

Fig. 1 showed the SEM images of bare CILE and 3D-GR modified CILE. On CILE a uniform and smooth surface appeared with graphite powder connected, which could be attributed to the presence of high viscous IL that adhered the graphite powder and fill the void space between them (Fig. 1A). Fig. 1B and C showed the SEM images of 3D-GR/CILE with different magnification, which exhibited a well-defined and interconnected 3D porous network. The result was in good agreement with the reported result [38]. Electrochemical reduction of GO to get GR is an efficient method for the preparation of GR modified electrode with green nature. By controlling the conditions for electrochemical reduction, the oxygenal groups of GO can be directly reduced with the recovery of the structure of GR and the formation of a 3D structure directly on the electrode surface [38,39]. From Fig. 1B it can be seen that few of porous and cavity-like structures were formed by GR sheets with the pore sizes in the range of several micrometers to larger than 10 μm. Furthermore, those pores were interconnected with each other to form the 3D-GR network, which could provide the largely exposed surface area and the quantity of edges sites of GR with high activity. The resulting 3D-GR network with high conductivity can also act as electron mediator of protein and are beneficial to the direct electrochemistry of redox protein. The close view of the 3D-GR structure shows some smaller pores and cavities inside the bigger ones (Fig. 1C). The hierarchical structure of pores/cavities is also helpful for the protein immobilization and direct electron transfer through the whole modified electrode.

The effective surface area of 3D-GR/CILE was further calculated by cyclic voltammetry in a 1.0 mmol/L $K_3[Fe(CN)_6]$ and 0.1 mol/L KCl solution. By changing the scan rate and recording