



Single layer of graphene/Prussian blue nano-grid as the low-potential biosensors with high electrocatalysis



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ABSTRACT

A single-layer nanogrid of thiol graphene/Prussian blue (tG/PB) composite film was constructed to realize the high electrocatalytic biosensing of various physiological substances under the low potential. In order to achieve this architecture, a single layer of deformed polystyrene (PS) beads was first prepared as the template for the PB deposition. The structure of PB film can be well controlled to build a nanogrid with uniform 500 nm cavities by the self-assembly approach. This film can capture the thiol graphene into its nanogrid cavities to form the composite nanostructure via a strong ionic bond between thiol group from the graphene and Au from the substrate. Both electrocatalysis and conductivity of the as-prepared tG/PB film can harvest the remarkable enhancements due to the synergic effects of PB and graphene. As demonstrated, biosensors constructed by this film exhibited the high sensitivities and accuracies in the electrocatalytic oxidation of glucose, lactate and glutamate under the very low potential -0.05 V.

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1. Introduction

Electrocatalysis of natural physiological substances takes a very important role in the development of bio-fuel cells [1,2] and biosensors [3]. No matter which application, challenges always happen at the high oxidation potential and low electrocatalytic current density of the working electrode. High overpotential will easily damage the modified film and arouse other side reactions from the co-existed substances [4], as well as the dissatisfied response power from the low current density. Biosensor was then developed to address above issue. Due to the specific oxidation of the matching physiological substance, the onset potential can be normally reduced with rare interference [5]. However, the applied enzymes are always low conductive proteins which possess the deeply wrapped redox active center (flavin adenine dinucleotide, FAD) by the peptide molecules [6]. In this case, the electrochemical redox electrons will be hindered for transfer, which strongly cuts the received current signal.

Recently, various nanomaterials including carbon [7,8], conducting polymers [9], metals [10–14] and metal oxides [15–20] have been used to functionalize the working electrode by their

high catalysis ability or good conductivity for the magnification of the electrocatalytic signal. Large number of studies proved that the film materials which are distributed by regularly nano- or micro-structure usually possess much higher performance than disordered structure [21,22]. Hence, the regular shape of materials in nanoscale gradually attracted more and more attentions in the fabrication of electrode film.

Prussian blue (PB), which is a derived porous metal-organic frameworks (MOFs) [23], is famed by its stability and high catalysis to H_2O_2 [24,25]. It is often served as a good electron transport mediator when bioelectrocatalysis happens at a low potential. However, on account of the fast formation rate, PB is always difficult to self-organize the regular nanostructure. Facing this problem, we have already developed a series of new in-situ preparation techniques [26–29] to achieve the synthesis of various regular nanostructured PB films. However, although these fabricated working electrodes can function with the high electrocatalysis at the low potential, the conductive resistance obviously enhanced due to increasing staking gaps caused by the regular crystals. Besides, PB is a typical semiconductor with ca. 1.42 eV band gap [30] which cannot efficiently transfer the catalyzed electrons to reduce its electrochemical performance.

Graphene is an excellent conductive material with high conductivity of $\sim 2 \times 10^3$ S/cm [31]. Importantly, it possesses the good biocompatibility and nontoxicity [32,33] to avoid the

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pollution of enzymes or physiological substances. Some works [34,35] have demonstrated that graphene owns the ability of direct electron transfer from the protein FAD. However, graphene is of a layered structure which easily self-assembles together as a very thick film with numerous sheets. If graphene is directly composited with PB, the surface of PB will be totally covered, which hinders the directly contact between the target and PB to strongly reduce the electrocatalysis of the prepared film. Therefore, a smart combination method and structure are required to produce the synergetic effect from the electrocatalysis of PB and conductivity of graphene.

In this work, we self-assembled a single-layer nanogrid structure of PB on the gold electrode for the positioning deposition of graphene. By using the thermal treatment to deform the polystyrene (PS) template, the formed PB grid can expose the substrate to provide the regular binding sites. A thiol grafted graphene (tG) was then deposited and captured into the cavity shaped by the PB grid through the strong ionic bond between thiol group (-SH) from the graphene and Au from the substrate. This special film architecture can combine the high catalysis with conductivity to bring about the high-performance in detection of glucose, lactate and glutamate at a very low potential -0.05 V. Furthermore, the produced current densities were still exhibited to a high level when the addition of very low concentrations of various physiological substances.

2. Experimental

2.1. Reagents and apparatus

Thiol graphene (2.5 mg/ml, dispersing in deionized water) were purchased from Henqiu Tech. Inc. Polystyrene latex microspheres (PS, 500 nm, 2.5 wt%) were purchased from Alfa-Aesar company. Uric acid and ascorbic acid were received from Sinopharm Chemical Reagent Co. Ltd. (China). $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ (Sigma-Aldrich), $\text{K}_4[\text{Fe}(\text{CN})_6] \cdot 3\text{H}_2\text{O}$ (Sigma-Aldrich), sodium n-dodecyl sulfate (Alfa-Aesar), (3-glycidyloxypropyl) trimethoxysilane (Sigma-Aldrich), glucose (Sinopharm Chemical Reagent Co., Ltd, China), L-glutamic acid monosodium salt monohydrate (Alfa-Aesar), sodium L-lactate (Alfa-Aesar), were all analytical grade purity and used without further purification.

5 mg glucose oxidase (GOD, EC 1.1.3.4, 168800 units per g, from *Aspergillus niger*, Sigma-Aldrich) was dispersed in 1 mL of 0.05 M phosphate buffer solution with 0.1 M KCl (PBS, pH = 6.5) and was sufficient for preparation of five electrodes. 1 unit glutamate oxidase (GMOD, EC 1.4.3.11, 5 units per mg, from *Streptomyces* sp., Sigma-Aldrich) was mixed into 100 μL PBS. 5 mg lactate oxidase (LOD, EC 1.13.12.4, 20 units per mg, from *Pediococcus* sp., Sigma-Aldrich) was dispersed in 2 mL PBS and separated into 20 separate vials (5 units per 100 μL). All solutions were prepared with deionized water.

Scanning electron microscopy employed a JEOL JSM-6390LV with a SC7620 Mini Sputter Coater from Quorum Technologies. Online monitoring of graphene coverage was fulfilled by quartz crystal microbalance. (QCM200, Stanford Research Systems, Inc., USA). The electrochemical properties were studied using an electrochemical workstation (CHI 660E, (Shanghai Chenhua, China)). Amperometry characterization experiments were operated in PBS at room temperature. Electrochemical impedance spectroscopy (EIS) measurements were performed in the presence of a stationary 5 mM $\text{K}_3[\text{Fe}(\text{CN})_6]/\text{K}_4[\text{Fe}(\text{CN})_6]$ (1:1) mixture as a redox probe in 0.1 M KCl solution with the frequency changed from 0.1 Hz to 1000 kHz with signal amplitude of 5 mV at the 0.05 V potential. A Pt wire and Ag/AgCl (saturated KCl) were used as the counter and reference electrodes, respectively. The scan rate in cyclic voltammetry was 50 mV s^{-1} .

2.2. Preparation of a PB single-layer grid

The Au disk electrodes (geometrical area ca. 3.14 mm^2) were polished with 1.0, 0.3 and 0.05 micron size alumina powder, and then the electrodes were sonicated in deionized water for 10 min and in ethanol for 10 min. Finally, the cleaned electrodes were dried at 60 °C.

After the above pre-treatment step, firstly, one or two drops of PS beads were added on the electrode surface in order to cover the whole Au area. The electrode was held stationary for 1 min in order to enable dispersion of the beads on the Au surface. Secondly, the substrate was slowly immersed into deionized water and one or two droplets of 2% sodium n-dodecyl sulfate were dropped into this water in order to change the surface tension. At this stage, the PS beads were dispersed and the single layer suspended on the water. Afterwards, the prepared single layer of PS modified electrode was lifted out of the trough and dried in the oven at 110 °C for 60 min in order to deform and fix the beads to the surface.

When the deposition of the PS single layer was finished, the electrode was modified with PB using a self-assembly approach. Two solutions were prepared for the deposition of PB films: solution 1 (S1): 0.01 M $\text{K}_4[\text{Fe}(\text{CN})_6]$ + 0.1 M KCl + 0.1 M HCl and solution 2 (S2): 0.01 M FeCl_3 + 0.1 M KCl + 0.1 M HCl. The electrode was dipped into the solutions in the following order: (1) S1 for 60 s; (2) deionized water for 15 s $\times$ 3 times for cleaning; (3) S2 for 60 s; and (4) deionized water for 15 s $\times$ 3 times. The above steps can be repeated in order to control the amount of PB on the electrode surface. The self-assembly time and operation were controlled accurately using a Dip-Robot (DR-3, Riegler & Kirstein GmbH, Germany) for the whole process. After the film was prepared, the electrode was washed and dried at 60 °C for 30 min. Finally, the prepared electrode was immersed in toluene for 36 h to remove the PS template and obtain a single layer of PB grid.

2.3. Deposition of thiol graphene

5 ml thiol graphene solution was added into 45 ml deionized water to form graphene deposition source. In order to uniform disperse the graphene sheets, this graphene dispersion was then vibrated by a probe-type sonicator (KBS-650, Kunshan Shumei, China) with a 20 KHz of ultrasonic frequency for 30 min. Subsequently, the modified electrode was vertically suspended to immerse the graphene source. After 250 seconds, the graphene film has been already modified on Au surface.

2.4. Immobilization of enzyme

The electrode was dipped into 2% (3-glycidyloxypropyl) trimethoxysilane (GS) at 60 °C for 1 h. The excess liquid was removed from the electrode surface and allowed to dry at 60 °C for 30 min. Finally, the electrode was immersed into the enzyme solution in order to allow enzyme binding (GOD for 3 h, GMOD and LOD overnight). Finally the electrode was washed using deionised water and stored in 4 °C.

3. Results and discussion

3.1. Formation of the PB grid

PB is a stable metal coordination complex without any active group to combine with graphene. The routine deposition method of PB and graphene could cause the masking of PB surface by graphene sheet to obviously reduce the catalysis, as well as the weak stability during the electrochemical tests because of the weak interaction between PB and graphene. Therefore, as shown in

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