Enzymeless electrochemical detection of hydrogen peroxide at Pd nanoparticles/porous graphene

Wanyi Xue, Xiangjie Bo *, Ming Zhou, Liping Guo *

Key Laboratory of Nanobiosensing and Nanobioanalysis at Universities of Jilin Province, Department of Chemistry, Northeast Normal University, 5268 Renmin Street, Changchun, Jilin Province 130024, PR China

A B S T R A C T

In this work, Pd nanoparticles/porous graphene (Pd/PGR) is prepared by simultaneous reduction of graphene oxide (GO) and Pd precursor in sodium borohydride ($NaBH_4$) solutions at the electrode surface. In this preparation, the positively charged ZnO with high isoelectric point is used as templates for the adsorption of negatively charged GO in water. Then, the poly(diallyldimethylammonium chloride) (PDDA) is added into the ZnO/GO through the electrostatic interaction between the negative charge of GO and positive charge of PDDA. Subsequently, PDDA/ZnO/GO is employed as a support material for the adsorption of chloropalladic acid ions ($PdCl_4^{2-}$), via the self-assembly between the negative Pd precursor and positively charged functional groups of PDDA. One-step reduction of Pd precursor/PDDA/ZnO/GO in $NaBH_4$ solutions and the subsequent removal of the ZnO produce Pd/PDDA/PGR with a porous structure at the electrode surface. With a porous morphology and large surface area for both efficient exposure of Pd nanoparticles and enhanced electrolyte-reactant diffusion, a sensitive enzymeless sensor of hydrogen peroxide (H_2O_2) is constructed. Compared with the nonporous Pd/PDDA/GR, the Pd/PDDA/PGR displays high electrocatalytic activity towards H_2O_2, exhibiting a high sensitivity of 57.7 $\mu A\ mM^{-1}$ and low detection limit of 0.9 μM towards the reduction of H_2O_2. The improved activity and simple preparation method makes Pd/PDDA/PGR promising for being developed as an attractive robust and new electrode material for electrochemical sensor and biosensor fabrication.

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1. Introduction

Hydrogen peroxide (H_2O_2) is widely used in waste-water treatment processes, clinical, paper-bleaching, and industrial manufacture [1]. In addition, H_2O_2 is also a byproduct of a large number of oxidase enzymes [2]. Therefore, sensitive detection of H_2O_2 is important in biomedical and environmental monitoring. The common-used techniques for H_2O_2 determination are chemiluminescence [3,4], fluorimetric method [5], and titrimetry [6]. Besides these approaches above, electrochemical sensors are also of particular interest for their practicality, simplicity, low-cost, and suitability for real-time detection [7–11]. The enzyme-based H_2O_2 biosensors generate high activity and selectivity [12,13]. However, due to the low stability and high cost of enzymes, the wide utilization of enzyme-based H_2O_2 biosensors is hindered. The replacement of enzyme-based H_2O_2 biosensors with inexpensive inorganic nanomaterials is a desired way to overcome the low stability and high cost of enzymes [7]. To achieve this goal, various inorganic nanomaterials, including carbon nanomaterials [14–16], metal oxides [17], noble metal nanoparticles [18–21], and their composites [16,22],

have been used as sensitive sensors for H_2O_2 with long-term stability and high sensitivity.

Among the above-mentioned carbon nanomaterials, graphene (GR) and its composites have been extensively used in electrochemical sensors because of excellent electrical conductivity, large theoretical specific surface area, and great mechanical strength [23–27]. In 2008, GR was first used as an electrode material for electrochemical sensing of dopamine (DA) [28]. In this work, GR-modified electrode was first demonstrated fast electron-transfer kinetics for DA and very good performance for selective determination of DA in the presence of ascorbic acid (AA) and uric acid (UA). Following the initial observation of high electrocatalytic performance from GR, the electrocatalytic activity of GR-based electrochemical sensors or biosensors have been widely reported and applied in various analytical fields such as environment monitor, food security, and disease diagnosis [26,27,29]. In addition to acting as electrode materials in electrochemical sensors, by preparing nanoparticles on GR support, the performance of GR-based composites can usually be dramatically enhanced. For example, Fe_3O_4 magnetic nanoparticles showed low activity towards H_2O_2, DA, and nitrite on their own; however, their activity is greatly improved when they were supported on GR support [30]. The Fe_3O_4/GR combined the high electrical conductivity of GR and high catalytic activity of Fe_3O_4, synergistically

* Corresponding authors.
E-mail addresses: baoxj133@nenu.edu.cn (X. Bo), guolp078@nenu.edu.cn (L. Guo).

enabling the high activity. Similar conclusions were arrived at for CeO_2/GR [31], TiN/GR [32], NiO/Ni/GR [33], and Cu_2O/GR composites [17]. Although many progresses have been achieved using GR as electrode materials or supports, GR sheets tend to stack together through π-π interaction [34,35]. Due to irreversible aggregation of GR layers, the surface of GR is then much negatively affected by the overlapping of sheets. The active sites for electrocatalysis are easily filled between GR sheets or hidden inside the stacked GR, then being absent for electroanalysis. After the aggregation, nanoparticles supported on GR layers are easily sandwiched between aggregated GR sheets or secluded inside the stacked layers and consequently the utilization efficiency of supported nanoparticles is limited. Then, the unique catalytic properties of nanoparticles are lost, such as high surface area and high density of active sites. Therefore, it is important to alleviate the aggregation of GR layers and increase the surface area of GR.

As discussed above, the structural properties of GR and utilization efficiency of supported nanoparticles are much affected by the aggregation of GR layers, which hinders its applications in electrochemical studies. Hence, GR with different structures have been used to alleviate the aggregation of GR layers and increase the surface area of GR, including vertical structure [36,37], tube-like structure [38], and porous structure [39–41]. The large distance between layers of nanostructured GR prevents the aggregation of GR sheets and then favors the exposure of anchoring sites for growth of nanoparticles. In addition, the unique porous properties of these GR nanostructures ensured efficient mass transport and also increased the electrochemical activity. Among these structurally engineered GR, porous GR (PGR)-based materials have been extensively designed, prepared and studied for the practical applications in supercapacitors, lithium-ion batteries, gas adsorption, electrochemical biosensors, and fuel cells [39–42]. To prepare the PGR, hard-template method [43,44], template-directed chemical vapor deposition (CVD) method [45], chemical oxidative etching method [46–48], and hydrogel or aerogel methods [49] are common strategies for the synthesis of PGR. Among these methods, hard-template method is an important and easy method for the preparation of PGR structures because of its controllable pore size [43,50]. Generally, polymer or inorganic spheres are used as hard templates for the self-assembly of GO onto their surface. Subsequent reduction of GO and removal of templates results in crumpled GR with pores that are originally occupied by the template species. Hard-templated PGR with interconnected porous structure has wide applications in energy conversion or environment pollution enrichment and separation, as emphasized in recent reviews [40,41].

In this work, a simple method is developed to preparation of Pd nanoparticles/PGR composites. Due to the high isoelectric point of ZnO (9.5) [51], the GO was first assembled on the surface of ZnO because of electrostatic interaction between the positive charge of ZnO and negative charge of GO. Then, the positive-charged poly(diallyldimethylammonium chloride) (PDDA) was added into the ZnO/GO solutions (PDDA/ZnO/GO). Subsequently, the negative-charged chloropalladic acid ions ($PdCl_4^{2-}$) was adsorbed on the PDDA via the self-assembly between the negative Pd precursor and positively charged functional groups of PDDA. One-step reduction of Pd precursor/PDDA/ZnO/GO in $NaBH_4$ solutions and the subsequent removal of ZnO produce Pd/PDDA/PGR with a porous structure on the electrode surface. Recently, the PGR prepared with different method has been reported as support for nanoparticles. For example, template-directed CVD is an important and easy method to prepare foam-like porous GR. Chen et al. first reported foam-like GR using an interconnected nickel scaffold as template [45]. Based on this pioneering work, it has been shown that macroporous, highly conductive, and monolithic GR foam synthesized by CVD can be used as support for nanomaterials in electrochemical sensors, including Co_3O_4 nanowires [52], ZnO nanowires [53], Mn_3O_4 nanomeshes [54], $NiCo_2O_4$ [55], CuO nanoflower [56], and $Ni(OH)_2$ nanosheets [57]. Although the CVD-grown GR foam is widely used in electrochemical sensors, GR foam prepared from CVD suffers from the involvement of an expensive machine. Besides the CVD method, hard-template method and GR hydrogels or aerogels are very common strategies for the preparation of PGR [40,41,49]. Additionally, porous Pd nanoparticles supported on GR also exhibited high activity towards the H_2O_2 [58]. However, large amount of reagent is required for the preparation of PGR and its composites. Although PGR has been reported as support for nanoparticles, our methodology is special because: (1) Pd/PDDA/PGR is prepared on the electrode surface by a simple method without involvement of substantial reagent; (2) The Pd loading amount can be easily adjusted by adsorption time of $PdCl_4^{2-}$. The combination of the unique properties of Pd nanoparticles and PGR endows Pd/PDDA/PGR as an electrochemical sensor for H_2O_2.

2. Experimental

2.1. Reagents and apparatus

PDDA (20 wt.% aqueous solutions, MW = 100.000–200,000) were purchased from Sigma-Aldrich. H_2O_2 and $PdCl_2$ were purchased from Sinopharm Chemical Reagent Co., Ltd. All other reagents used were of analytical grade and used as received without further purification. The 0.1 M phosphate buffer saline (PBS, pH 7.4) was employed as a supporting electrolyte. For analysis of the real sample, human serum samples were obtained from the attached hospital of Northeast Normal University and subjected to ultrafiltration at 5000 rpm. Then, the serum samples were diluted 5 times with PBS (pH 7.4) and different concentrations of H_2O_2 were spiked to these samples.

The X-ray diffraction (XRD) pattern was obtained on an X-ray D/max-2200vpc (Rigaku Corporation, Japan) instrument operated at 40 kV and 20 mA using Cu Kα radiation (k = 0.15406 nm). Electrochemical tests were performed on a CHI 660C electrochemical workstation (CH Instruments, China). A three-electrode configuration was employed, consisting of a modified glassy carbon (GC, 3 mm diameter) electrode serving as a working electrode, whereas an Ag/AgCl (in saturated KCl solutions) and a platinum wire served as the reference and counter electrodes, respectively. Electrochemical impedance spectroscopy (EIS) was conducted using a Par 2273 Potentiostats-Electrochemistry Workstation in 5 mM $Fe(CN)_6^{4-/3-}$ and 0.1 M KCl solutions at +0.25 V from 0.1 Hz to 10.0 KHz. Relative to Ag/AgCl reference electrode, the redox probe of $Fe(CN)_6^{4-}$ and $Fe(CN)_6^{3-}$ exhibits a pair redox peaks with formal potential of around +0.25 V at the electrode surface. This potential is near the formal potential of $Fe(CN)_6^{4-/3-}$ pair, and makes the redox rates equal. Philips XL-30 ESEM equipped with an energy-dispersive X-ray spectrometer was used to determine the morphology and component of products. X-ray photoelectron spectroscopy (XPS) was measured using Thermo ESCA LAB spectrometer (USA).

2.2. Preparation of Pd/PDDA/PGR

ZnO spheres were prepared according to previous method in the literature [59]. GO was synthesized according to modified Hummers' method [60]. The preparation way of Pd/PDDA/PGR is presented in Scheme 1. 1.6 mg of ZnO particles were first mixed with 1 mL of GO
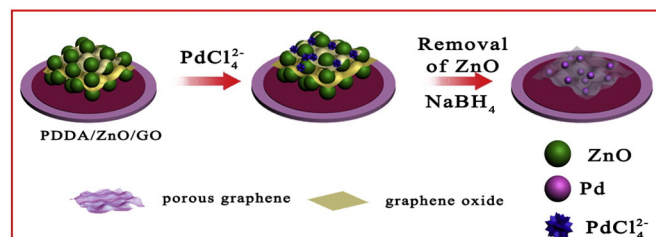
Scheme 1. Preparation pathway for Pd/PDDA/PGR.