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Horseradish peroxidase supported on porous graphene as a novel sensing platform for detection of hydrogen peroxide in living cells sensitively

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ABSTRACT

A viable and simple method for preparing porous graphene network using silver nanoparticles (AgNPs) etching was proposed, and a sensitive biosensor was constructed based on the porous graphene (PGN) and horseradish peroxidase (HRP) to measure the release of H_2O_2 from living cells. Owing to the large surface area and versatile porous structure, the use of nanoporous materials can significantly improve the analysis performance of the biosensor by loading large amounts of enzyme and accelerating diffusion rate. Meanwhile, the constructed electrode exhibited excellent electrochemical performance toward H_2O_2 with a determination limit as low as 0.0267 nM and wide linear range of 7 orders of magnitude, which was superior to other H_2O_2 electrochemical sensors. Thus, this novel biosensor can detect the H_2O_2 release from living cells not only under normal physiological conditions (10^{-8}–10^{-7} M) but also in emergency state with the increased concentration ($\sim 10^{-4}$ M). This work provides tremendous potential for real-time tracking of the secretion of H_2O_2 in different types of physiological and pathological investigations.

1. Introduction

Reactive oxygen species (ROS) are important intracellular signaling molecules, mainly regulating protein synthesis, DNA damage, cell apoptosis, etc. (Chang et al., 2013). Nevertheless, the excessive amount of ROS accumulation in cells could lead to a series of cell damage and cause aging and disease, including cardiovascular disease, Alzheimer's disease and cancer (Pagliari et al., 2012; Wu et al., 2011). H_2O_2, a common representative of reactive oxygen species (ROS), has received much consideration since its long lifetime allows it to penetrate into other cellular compartment to induce various harmful biological modifications potentially. It is closely related to people's safety and health (Zhang et al., 2013; Trachootham et al., 2009). Hence, developing a rapid, sensitive, and accurate method to measure H_2O_2 dynamic release process from living cells is essential in studying the biological effect of H_2O_2 and preventing relative diseases associated with human inflammatory (Rhee et al., 2010; Zhang et al., 2014a; Maji et al., 2014; Oh et al., 2012).

Compared with fluorometry, spectrophotometry, and chromatography, electrochemical methods are more effective for in situ and real-time analysis of H_2O_2 due to their fast response, good selectivity, high sensitivity, excellent reproducibility, and facile operation (Yuan et al., 2012; Xu et al., 2014; Xi et al., 2015). In particular, electrochemical methods based on the catalytic reduction of H_2O_2 by the natural enzyme, for example, horseradish peroxidase (HRP), have witnessed an increasing interest because of their high efficiency, good selectivity and sensitivity toward H_2O_2 (Chen et al., 2016; Feyzizarnagh et al., 2016). However, the immobilization and stabilization protocol of the enzyme on the electrode are complicated, and the activity of the enzyme electrode is easy to reduce (Lippert et al., 2011). Moreover, due to the low concentration of H_2O_2 in cell, the detection limit still needs to be improved. In order to solve these problems, it is necessary to develop the excellent support matrix that provide better environment for loading the enzyme efficiently and maintaining the enzymatic bioactivity.

Porous graphene, the isolated two-dimensional carbon nanomaterial with some holes/pores within the atomic plane, has attracted great attention recently in the fields of nanomaterial and electrochemistry on account of its extraordinary physiochemical properties including larger surface area, higher electron conductivity and better biocompatibility (Zhou et al., 2014; Xi et al.,

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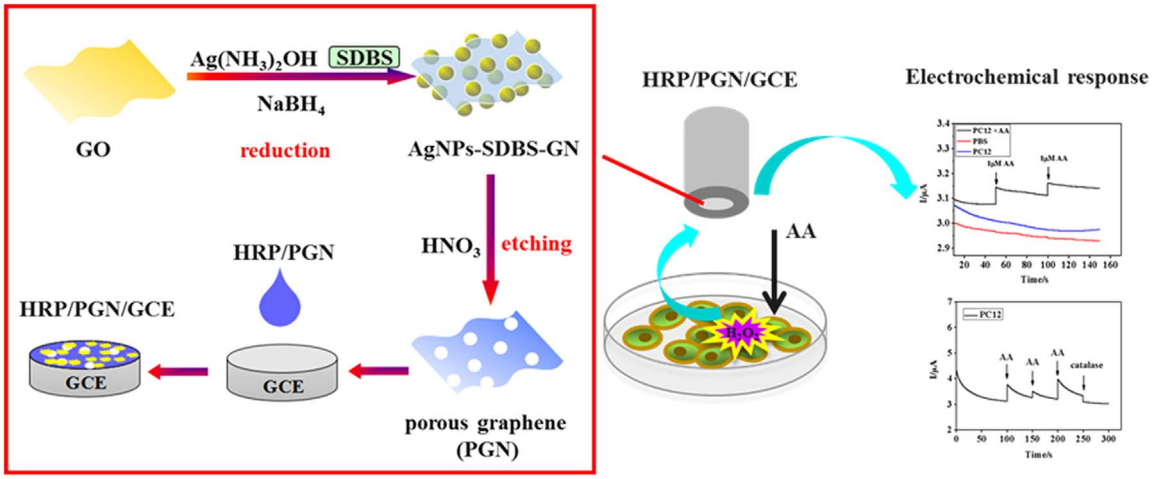

Scheme 1. Schematic of the HRP/PGN modified GCE used for detecting H_2O_2 release from cells stimulated with ascorbic acid (AA).

2012). Moreover, construction of graphene into porous structure can effectively prevent aggregation of graphene sheets (Han et al., 2014; Jiang and Fan, 2014), and is beneficial of the adsorption of enzyme. Meanwhile, nanopores are advantageous for ions passing through (Sint et al., 2008). These merits made porous graphene (PGN) an excellent supporting material. However, little work is available on the application of enzyme/porous graphene as electrochemical sensors.

In this work, we developed a novel enzymatic H_2O_2 sensor based on PGN and HRP to measure the release of H_2O_2 from living cells (Scheme 1). As demonstrated later, such a biosensor can facilitate the electron transfer of HRP and improve analytical performance for the determination of H_2O_2. Most importantly, the outstanding features of PGN, combined with the excellent selective catalysis of HRP, enable this biosensor to be used for determining the release of H_2O_2 from living cells with satisfaction.

2. Material and methods

2.1. Apparatus

The transmission electron microscopy (TEM) images were obtained from JEM-3010 transmission electron microscope (JEOL Co., Ltd., Japan). Raman spectra were recorded on a Renishaw inVia Raman microscope system with 632.8 nm laser excitation. Nitrogen sorption isotherms were obtained with a Micromeritics TriStar II 3020 surface area and porosity analyser at 77 K. The samples were degassed overnight at 110 °C. The Brunauer–Emmett–Teller (BET) method was used to calculate the specific surface area. Electrochemical measurements were performed on a CHI660C electrochemical workstation (Austin, TX, USA) using a three-electrode system with a glassy carbon electrode (GCE, d = 3.0 mm) or modified GCE as the working electrode, a saturated calomel electrode (SCE) and a platinum electrode as the reference and counter electrode, respectively. All potentials given in this paper were referred to the SCE. Before each electrochemical measurement, solutions were thoroughly deoxygenated by bubbling nitrogen through the solution for at least 20 min to remove dissolved oxygen.

2.2. Reagents

Graphite (99.99% SP-1, Bay carbon) with average particle size of 45 μm was obtained from Shanghai Chemical Reagent (Shanghai, China). Hydrogen peroxide solution (30 wt%) and nitric acid (HNO_3) were purchased from Beijing Chemical Reagent (Beijing, China). Silver nitrate ($AgNO_3$) and ammonia ($NH_3 \cdot H_2O$) were bought from Xi'an Chemical Reagent (Xi'an, China). Sodium dodecyl benzene sulfonate (SDBS) and sodium borohydride ($NaBH_4$) were obtained from Tianjin Chemical Reagent (Tianjin, China). Horseradish peroxidase (HRP) and ascorbic acid (AA) were obtained from Sigma. The catalases (2000–4000 units/mL) was purchased from Beijing Chemical Reagent (Beijing, China). A 0.2 M phosphate buffer solution (PBS, pH 7.0) comprising NaH_2PO_4 and Na_2HPO_4 was used as the supporting electrolyte. A physiological PBS solution containing KH_2PO_4 (1.76 mM), Na_2HPO_4 (10.14 mM), NaCl (136.75 mM), and KCl (2.28 mM), was mainly used for washing of PC12 cells and observing the release of H_2O_2 from the cells. Other chemicals were all of analytical grade, and the solutions were prepared by doubly distilled water.

2.3. Preparation of the porous graphene

Graphene oxide (GO) dispersions were prepared from the graphite powder by a modified Hummers' method (Li et al., 2008). The AgNPs-graphene nanocomposites were carried out by a facile and versatile hydrothermal synthetic strategy and the detailed preparation steps are as follows: as-prepared graphene oxide (0.05 g) was initially dispersed into 50 mL water under ultrasonic for 3 h, and SDBS (0.05 g) was dissolved into 20 mL water. Then SDBS solution was added into aqueous GO dispersion followed by approximately 60 min of ultrasonication to achieve the SDBS-GO suspension. $NH_3 \cdot H_2O$ (3%) was dropped into aqueous $AgNO_3$ solution (0.02 M, 10 mL) continuously until the precipitates disappeared and the Tollens' reagent was obtained. Next, the Tollens' reagent was dropped into as-synthesized SDBS-GO suspension and adequately stirred for 3 h. Following this, 70 mL of $NaBH_4$ (40 mM) solution was added into the above mixture at a stirring rate of 600 rpm for 12 h at 90 °C. The solution was filtered by nylon membrane with 0.22 μm pores, thoroughly washed with water to remove the free materials in the solution. The obtained black product, named as AgNPs-graphene (AgNPs-GN), was immersed in aqueous HNO_3 solution (1.0 M) and stirred for 3 days to remove AgNPs. Finally, the porous graphene (PGN) sample was collected by filtration and washed with water and ethanol. The product was obtained through freeze drying process.

2.4. Preparation of the H_2O_2 sensor

Prior to the modification, the glassy carbon electrode (GCE) was polished with 1.0, 0.3 and 0.05 μm alumina slurry to a mirror-like,