



Graphene oxide-gold nanozyme for highly sensitive electrochemical detection of hydrogen peroxide

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ABSTRACT

We fabricated a nafion/graphene oxide-gold nanoparticle (GO-AuNP) hybrid modified indium tin oxide (ITO) electrode and proposed an electrochemical method to detect hydrogen peroxide (H_2O_2) using 3,3,5,5-tetramethylbenzidine (TMB) as a redox mediator. The GO-AuNP hybrids were employed as nanozymes, which function as peroxidase mimics and show highly effective catalytic activity. Based on the high catalytic activity, enzyme mimics were entrapped on the ITO electrode to construct an electrochemical H_2O_2 sensor by coating nafion polymer. During the catalytic reaction, the peroxidase substrate TMB was oxidized to form the TMB oxidation product, which not only produces a blue color detected by absorbance change, but also generates an electrochemical current. As a result, both spectrophotometric and electrochemical methods were used to determine H_2O_2 concentration. The spectrophotometric detection displayed a linearity for H_2O_2 concentration from 10 μM to 5 mM ($r^2 = 0.989$), with an estimated detection limit of 2 μM . In the electrochemical detection, the TMB peak current had a good linear relationship with H_2O_2 concentration from 10 nM to 10 mM, with an estimated detection limit of 1.9 nM, which was much lower than that of the spectrophotometric method result.

1. Introduction

Nanozymes, a term invented by Scrimin, Pasquato, and co-workers, are nanomaterials that possess enzyme-like characteristics [1,2]. These artificial enzymes have attracted a lot of attention in various applications including target cancer therapy [3], diagnostic medicine [4], immunoassay [5], chemical industry [6], and environmental science [7,8]. Natural enzymes are mostly composed of proteins made from amino acid and can be easily denatured due to environmental conditions, leading to an inactive state [9]. Furthermore, their preparation, purification, and storage are quite difficult, time-consuming, and expensive [10]. However, nano-sized enzyme mimics have advantages of high stability as well as large scale synthesis, cost-effectivity, size and shape controllability, and ease of preparation and purification [11].

Graphene oxide (GO), the single-layer form of graphite, has received great attention due to its many unique properties, such as high carrier mobility, large surface area, high thermal conductivity, excellent mechanical stiffness, and good biocompatibility [12,13]. Despite its short history, these unique properties facilitate GO applications in various fields including high speed transistors [14], optoelectronic devices

[15], biosensors [16,17], biomedical fields [18], biomimetics [19], and heterogeneous catalysis [20]. Among these various applications, GO has been widely used for detecting hydrogen peroxide (H_2O_2) because its intrinsic peroxidase activity can catalyze the oxidation of 3,3,5,5-tetramethylbenzidine (TMB), which is a peroxidase substrate in the presence of H_2O_2 . Song et al. discovered that carboxyl-modified GO shows intrinsic peroxidase-like activity, and applied it to colorimetric detection of glucose [21].

Although gold (Au^+) is considered chemically inert, gold nanoparticles (AuNPs) have been found to exhibit peroxidase-like catalytic activity [22]. However, the potential of AuNPs as peroxidase mimics is confined by their relatively moderate catalytic activities and stability [23]. Their effectivity could be strengthened in comparison with single-component nanomaterial by combining them with other functional nanomaterials such as GO [13]. Gao et al. reported that graphene oxide-gold nanocluster hybrids showed a highly synergetic effect in catalytic activity. During the catalytic reaction, the oxidized form of TMB produces a blue color, and the reaction can be measured by absorbance change at 652 nm [24]. The catalytic reaction can be also measured to detect the TMB oxidation product, which is electroactive, thus allowing

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electrochemical detection. Many studies have employed colorimetric detection to measure peroxidase activity, while electrochemical detection has not been well developed. Compared to colorimetric detection, the electrochemical method can be employed to determine H_2O_2 with a lower detection limit; it is also fast, inexpensive, and portable with potential for miniaturization.

In this study, we proposed an electrochemical detection method with a nafion/GO-AuNP hybrid modified indium tin oxide (ITO) electrode using TMB as the electrochemical substrate for H_2O_2 determination. The GO-AuNP hybrids were produced by a simple method and used as nanozymes entrapped in the electrode under a nafion film. The composed GO-AuNPs could catalyze the oxidation of TMB by H_2O_2 , generating electrochemical signals and exhibiting more enhanced peroxidase-like activity than AuNPs and GO alone. Furthermore, evaluations of colorimetric and electrochemical methods, including detection limit and sensitivity, were compared. Consequently, under the optimized conditions, the electrochemical detection method exhibited significantly low detection limit and high reliability and reproducibility.

2. Experimental

2.1. Materials

GO solution was purchased from Graphene Square Inc. (Seoul, South Korea). ITO glass was obtained from Samsung Corning Advanced Glass (Asan, South Korea). Buffer solution composed of potassium hydrogen phthalate and sodium azide in deionized (DI) water was purchased from Samchun Chemical (Pyeongtaek, South Korea). Gold (III) chloride trihydrate ($\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$), 3,3',5,5'-tetramethylbenzidine (TMB), $\text{K}_3\text{Fe}(\text{CN})_6$, KNO_3 , a nafion solution (5 wt% in 15–20% water/lower aliphatic alcohols), dimethylformamide, H_2O_2 , and sodium citrate tribasic dihydrate were obtained from Sigma-Aldrich (St. Louis, MO, USA). Aqueous solutions were prepared with DI water.

2.2. Synthesis and characterization of GO-AuNPs

AuNPs were deposited on the GO sheet by a previously reported method with slight modification [25]. The GO solution was sonicated for 1 h before the synthesis reaction. Then, 15 mL of 0.8 mM HAuCl_4 solution was mixed with 1.25 mL of 1 mg mL^{-1} GO solution, followed by incubation at room temperature for 20 min. The mixture was heated to 80°C , and then 470 μL of 85 mM sodium citrate solution was added dropwise. After incubation for 1 h, the mixed solution was centrifuged for 15 min at 5000 rpm. The supernatant was discarded, and the precipitant was washed two times with distilled water to eliminate free AuNPs. The concentration of GO-AuNP hybrids was estimated to be 0.13 mg mL^{-1} . The morphology and structure of the synthesized nanozyme were characterized with atomic force microscopy (AFM, Veeco Instruments, Inc., NanoScope IV, Santa Barbara, CA).

2.3. Preparation of nafion/GO-AuNP hybrid modified ITO electrode

To prepare the nafion/GO-AuNP hybrid modified ITO electrode, 50 μL of GO-AuNP hybrid solution was dropped onto the surface of the ITO electrodes and incubated for 12 h at 4°C . After incubation, 5 μL of 2% nafion solution was cast onto the surface of the GO-AuNP/ITO electrode. Then, the modified electrode was dried at 4°C for 12 h, followed by washing with DI water and gently drying with nitrogen gas. The morphological changes of the modified electrode were investigated by field emission-scanning electron microscopy (FE-SEM, Hitachi Co. Ltd., S-4800, Tokyo, Japan).

2.4. Colorimetric measurements

The catalytic activity of the nanozymes was verified *via* spectrophotometric measurement. The UV/vis spectra were recorded with an

Optizen 2120 UV-vis spectrophotometer (Mecasys Co. Ltd., Daejeon, South Korea). The catalytic reaction was carried out in pH 4.0 buffer with 500 μM TMB and various concentrations of H_2O_2 as substrates. Then, 50 μL of the synthesized GO-AuNPs was employed as enzyme mimics with a final reaction volume of 1 mL. Then, the mixed reaction solution was incubated at room temperature for 10 min.

2.5. Electrochemical measurements

Electrochemical measurements were carried out with a CHI600C electrochemical analyzer (CH Instruments, Inc., Texas, USA) controlled by a personal computer. A three-electrode system was employed to perform the electrochemical analysis. The nafion/GO-AuNP modified electrode was used as the working electrode (WE), and a clean platinum wire and Ag/AgCl (saturated in 3 M NaCl) were used as the counter electrode (CE) and the reference electrode (RE), respectively.

3. Results and discussion

A schematic illustration of the synthesis of GO-AuNP hybrids is shown in Fig. S1. Exhibiting a stable dispersion in water, the GO suspension contains carboxyl, epoxy, carbonyl, and hydroxyl groups on the surface. These functionalities can lead to attachment of free gold ions onto the surface of GO *via* electrostatic interaction during the aging step. Then, the addition of the reducing agent, sodium citrate, advances the reduction of gold ions, resulting in the formation of AuNPs on the surface of GO sheets [26]. The deposition of AuNPs onto GO was characterized by AFM studies. Generally, the average thickness of single-layer graphene is around 0.8 nm. However, in Fig. 1A, GO flake thickness was about 1.3 nm measured from the height profile (thickness = average height – average baseline), due to the presence of the oxygen-containing groups on both sides of the GO surface. Fig. 1B shows the AFM image of the GO-AuNPs hybrids. Uniformly distributed bright spheres were located on the plane sheet, confirming that AuNPs were successfully assembled on the GO surface. The measured diameter of the grown AuNPs was around 25 nm.

The as-prepared GO-AuNPs hybrids could catalyze the oxidation of the peroxidase substrate TMB in the presence of H_2O_2 to produce a blue charge-transfer complex measured by absorbance at 652 nm (Fig. 2). The peroxidase-activity of the nanozyme was monitored by spectrophotometric measurement. Since the nanozymes are generally dependent on pH, like natural peroxidases, the catalytic activity of nanozymes was evaluated in the pH range of 3.0 to 8.0 (Fig. 3A). The highest catalytic activity was observed at pH 4.0, whereas activity decreased at neutral pH. Therefore, we selected pH 4.0 as the standard condition for the subsequent experiments. Fig. 3B presents the dependence of the absorbance at 652 nm on H_2O_2 concentration. Under the optimum pH, the absorbance at 652 nm increased with increasing H_2O_2 concentration. H_2O_2 could be detected as low as 2 μM , with a linear range from 10 μM to 5 mM ($r^2 = 0.989$) (Fig. 3C).

To investigate the synergetic effect of GO-AuNPs hybrids, the catalytic activity of single and hybrid materials was measured through the electrochemical detection method using TMB as the electrochemical substrate in the presence of H_2O_2 . TMB has been proposed to generate an electroactive product, allowing electrochemical detection in the presence of H_2O_2 and enzymes [27]. Fig. 4 shows comparison of the catalytic activity of AuNPs, GO, and GO-AuNPs based on current variation. The current variation means the change in the peak current of TMB between the absence and the presence of 1 mM H_2O_2 . With the increasing amount of oxidized TMB during the catalytic reaction, the oxidation peak current of TMB decreases. Therefore, an increment in current variation implies that a catalytic reaction occurs. At the same substrate concentration, GO-AuNP hybrids exhibited considerable changes in current, while AuNPs and GO showed relatively low current variation. From this result, we inferred that the enhancement of catalytic activity was ascribed to the synthesis of hybrid material due to the

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