Colorimetric H₂O₂ biosensor based on reduced graphene oxide-Hemin-Pt@Pd nanocomposites

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Abstract: Hydrogen peroxide (H₂O₂) is one of the most universal and essential ingredients in distinct biological tissues. Herein, a highly sensitive TMB colorimetric H₂O₂ biosensor was constructed based on the good catalytic activity of Hemin-reduced graphene oxide-Platinum@Palladium nanocomposites (H-rGO-Pt@Pd NCs). Colorless 3,3′,5,5′-tetramethylbenzidine (TMB) can be oxidize to blue product (TMB Ox) when H-rGO-Pt@Pd NCs catalyzes H₂O₂ reduction. The content of H₂O₂ can be obtained by spectrophotometric determination of the absorbance of the solution. In the range of 0-50 µM, the absorbance is linearly related to the H₂O₂ concentration, the detection limit is estimated to be 0.96 µM in terms of the rule of three times standard deviation over the blank with a correlation coefficient of 0.99264. The TMB colorimetric H₂O₂ biosensor shows that H-rGO-Pt@Pd NCs has good enzyme-like properties, which can greatly improve the sensitivity, stability and repeatability of the enzyme-free sensor, and has a great potential in biosensors.

1. Introduction

Hydrogen peroxide (H₂O₂) acts as a signal factor to regulate various biological processes and is a by-product of various oxidase reactions. H₂O₂ is one of the most common but important molecules existing in different biological tissues, and is of vital significance to human health and life [1]. At present, there are many methods for measuring H₂O₂ content, such as spectrofluorescence method, chemiluminescence method and electrochemical method [2]. Among them, colorimetric biosensor assays with exclusive advantages of visual recognition, simple operation and ultraviolet-visible spectrometer (UV-vis) quantification has the very broad application prospects in the field of clinical diagnosis [3-4]. However, for the natural enzyme are expensive, unstable, difficult to immobilization and easy to inactivation, the enzyme-based H₂O₂ biosensors limit their further application. So it is necessary to develop the peroxidase mimics and non-enzymatic H₂O₂ biosensors.

Recently, the materials with peroxidase-like activity have attracted great attention of researchers. Noble metal nanomaterial such as Au NPs, Pt NPs, Pd NPs have been witnessed a persistent utilization in electrochemical biosensor for their excellent conductivity, good biocompatibility, discrete electronic energy and excellent electrocatalytic activity towards the reduction of H₂O₂. Especially, Pt–Pd bimetallic NPs exhibits the superiority of outstanding intrinsic catalytic capacity, inherent biocompatibility and larger specific surface area over the corresponding monometallic NPs in virtue of the synergetic effect [5]. Reduced graphene oxide (rGO), as a deoxidized product of graphene oxide (GO), is also one of the most popular candidates to catalyze the electrochemical reduction of H₂O₂ [6]. Also, rGO provides a rich site for adsorption and immobilization of substances and can increase the adsorption rate. Hemin (H) is an iron porphyrin derivative and displays electrocatalytic abilities.
towards H$_2$O$_2$ based on the redox reaction of iron in the core [7].

In this work, a simple and sensitive TMB colorimetric H$_2$O$_2$ biosensor based on Hemin-reduced graphene oxide-platinum/palladium nanocomposites (H-rGO-Pt@Pd NCs) was introduced. H-rGO-Pt@Pd NCs has the peroxidase-like activities by the synergistic effect of Pt@Pd NPs, rGO and Hemin. H-rGO-Pt@Pd NCs catalyzed the H$_2$O$_2$-mediated oxidation peroxidase substrate TMB, resulting a color change. The colorless TMB was oxidized into blue oxidized TMB in the presence of H$_2$O$_2$ by the assistance of H-rGO-Pt@Pd NCs. The content of H$_2$O$_2$ can be obtained by spectrophotometric determination of the absorbance of the solution.

2. Experimental

2.1. Reagents and apparatus

GO was purchased from Xianfeng NANO Materials Tech Co., Ltd. (Nanjing, China). 3,3',5,5'-tetramethylbenzidine (TMB), ascorbic acid (AA), H$_2$O$_2$, Na$_2$PdCl$_4$ and Na$_2$PtCl$_4$ were obtained from Aladdin Reagents Ltd (Shanghai, China). All other analytical reagents used in the experiment without further purification. All the solutions were prepared with ultrapure water of 18 Ω·cm purified from a Milli-Q purification system (Milli-Pore, Bedford, MA, USA). Spectrophotometry were performed on an UH5300 UV-Visible spectrophotometer (Hitachi High Technologies Corporation, Japan). X-Ray Diffraction were performed on a D8 ADVANCE X ray diffractometer (Brock Company, Germany). Transmission electron microscopic (TEM) characterization was carried out on a JEM-1200EX transmission electron microscope (JEOL, Japan).

2.2. Synthesis of the H-rGO-Pt@Pd NCs

rGO was fabricated by using ascorbic acid and GO according to a route reported previously [8]. 30mg hemin was dissolved with 20μL NH$_3$·H$_2$O to form a clear solution by intense stirring, followed by the addition of 20μL 1mg/mL rGO solution that was stirred for 30 min. then added 5μL hydrazine hydrate (N$_2$H$_4$·H$_2$O) and stirred at 50℃ for 2 h to obtain H-rGO solution. After that, 2 mL PDDA (wt = 2%) and 2 mL NaCl (wt = 2%) were added to the H-rGO solution. After stirring for 12 h, a PDDA-modified H–rGO solution was obtained through centrifugation. Subsequently, 2 mL 20 mmol/L Na$_2$PdCl$_4$ and 2 mL 20 mmol/L Na$_2$PtCl$_4$ were added to the PDDA-modified H–rGO (0.5 mg/mL) solution. The mixture was added with 10.0 mL of glycol solution and 1.0 mol/L NaOH to adjust the pH to 12 with stirring and reacted at 140℃. After stirring for 4 h, the resulting black products were collected by centrifuging for 10 min at 12000 r/min and then washed with water several times. Subsequently, the yielded H-rGO-Pt@Pd NCs were freeze-dried for 16 h.

2.3. Detection of H$_2$O$_2$ with the TMB colorimetric biosensor

First, 40 μL 5.0 mg/mL of H-rGO-Pt@Pd NCs was added to 60 μL 125 mmol/L PBS buffer solution (pH 5.0), and then 20 μL 50 mmol/L of TMB was added. Third, 20 μL different concentrations of H$_2$O$_2$ solutions was added and incubated for 30 min at 25°. Finally, the resulting reaction products solution was measured with a UH5300 ultraviolet-visible spectrophotometer. The absorbance intensity at 652 nm was record. The peak value was positively correlated with H$_2$O$_2$ concentration.

3. Results and discussion

3.1 Characterization of H-rGO-Pt@Pd NCs

UV characterization of H-rGO-Pt@Pd NCs was shown in Figure 1A, the characteristic peaks of RGO and Hemin are at 260 nm and 367.5 nm, respectively. The XRD characterization of H–rGO-Pt@Pd NCs is shown in Figure 1B. There are sharp diffraction peaks at 2θ=39.76°, 46.24°, and 67.45°, which are correspond to (111), (200), (220) of Pt and Pd. rGO has a weak broad diffraction peak at 2θ≈22°, which belongs to the diffraction surface of graphene material C (002). The above phenomena indicate that the H–rGO-Pt@Pd NCs was successfully prepared.
Figure 1C is the TEM image of H-rGO and Figure 1D is the TEM images of H-rGO-Pt@Pd NCs. It can be clearly seen that there are many fine particles embedded on the H-rGO composite of thin film yarn mesh. This indicates that Pt NPs and Pd NPs have been well combined with H-rGO materials, and it can be concluded that H-rGO-Pt@Pd NCs materials have been successfully prepared.

3.2 Principle of colorimetric H2O2 biosensor based on H-rGO-Pt@Pd NCs

The principle of the TMB colorimetric H2O2 biosensor based on H-rGO-Pt@Pd NCs was schematically shown in Figure 2A. First, add H-rGO-Pt@Pd NCs nanoenzyme and TMB to the PBS buffer solution, then different concentrations of H2O2 was added to the above solution. H2O2 is decomposed to H2O and O2 under the catalysis of H-rGO-Pt@Pd NCs nanoenzyme, while TMB converts to oxTMB in the presence of O2. The solution changes from colorless to blue, and the absorbance values of the catalyzing TMB−H2O2 reaction products were recorded through an UV-vis photometer. The absorbance intensity at 652 nm was proportionally increased owing to the fact that TMB can be oxidized by H-rGO-Pt@Pd NCs for the production of the blue oxidized product (TMBox). Accordingly, the quantitative detection of H2O2 can be realized in accordance with the change of the colorimetric signal in the system.

Figures 2B and Figures 2C show the feasibility of experimental phenomena and absorbance graph of verifying the intrinsic oxidase-mimicking catalytic potential of H-rGO-Pt@Pd NCs, respectively. From Figure 2B, it can be seen that tube a contains H2O2 and does not decompose in air. Therefore, there is no absorbance. Tubes b–e are light blue and have lower absorbance, this is because TMB will be partially oxidized in the presence of oxygen in the air. While tube f has the darkest blue color and has the highest absorbance. Because H-rGO-Pt@Pd NCs nanoenzyme has catalase-like catalytic activity, which can catalyze the oxidation of TMB by H2O2 to make the solution produce color changes that can be observed and resolved by naked eyes.
Figures 2 (A) Schematic diagram of colorimetric H$_2$O$_2$ biosensor based on H-rGO-Pt@Pd NCs; (B) Feasibility experiment picture of H$_2$O$_2$ colorimetric biosensor based on H-rGO-Pt@Pd NCs; (C) Absorbance of feasibility of TMB colorimetric H$_2$O$_2$ biosensor based on H-rGO-Pt@Pd NCs

3.3 Stability analysis of H-rGO-Pt@Pd NCs

To explore different storage temperature and time of H-rGO-Pt@Pd NCs simulation the effect, the stability of H-rGO-Pt@Pd NCs were investigated. H-rGO-Pt@Pd NCs was divided into two portions, one for under 4℃ in the refrigerator, and another at room temperature (25℃), and the catalase-like catalytic activity was tested using the TMB-H$_2$O$_2$ chromogenic system in the time of 0 day, 1 day, 3 days and 7 days.

Figure 3A showed the experimental phenomenon of the influence of different storage temperature and storage time on the stability of H-rGO-Pt@Pd NCs. It can be seen from Figure 3A that all the solutions of TMB-H$_2$O$_2$ chromogenic system change from colorless to blue. H-rGO-Pt@Pd NCs has relatively good stability and can be stored for a long time. Through the color comparison between No. 2 and No. 3, No. 4 and No. 5, No. 6 and No. 7, we can know that H-rGO-Pt@Pd NCs is more suitable for low temperature preservation.

Figure 3B and Figure 3C showed the UV-Vis absorption spectra of the effect of different storage times on the stability of H-rGO-Pt@Pd NCs under 4℃ and 25℃. The absorbance intensity of curves 1-7 at 652 nm was $1 > 3 > 2 > 5 > 4 > 7 > 6$. The results showed that H-rGO-Pt@Pd nanozyme has certain stability, but its catalase-like catalytic activity will decrease with the time. By comparing curves 2 and 3, 4 and 5, 6 and 7, we can know that H-rGO-Pt@Pd NCs is more suitable for cryopreservation.
Figure 3 (A) Experimental diagram of the effect of storage temperature and time on stability H-rGO-Pt@Pd NCs. (B) UV-Vis absorption spectra of the effect of different storage times on the stability of H-rGO-Pt@Pd nanozyme under 25℃. (C) UV-Vis absorption spectra of the effect of different storage times on the stability of H-rGO-Pt@Pd nanozyme under 4℃.

3.4 Performance analysis of H2O2 colorimetric biosensor based on H-rGO-Pt@Pd NCs

Under the optimal conditions, the H2O2 colorimetric biosensor based on H-rGO-Pt@Pd NCs was used to detect H2O2. The results are shown in Figure 4. Figure 4A showed the color change of the solution at different H2O2 concentrations. It can be seen that the higher of H2O2 concentration was, the darker color had, which demonstrated that the colorimetric biosensor could be put to use for detecting H2O2 with naked eyes. As shown in Figure 4B, the absorbance intensity at 652 nm increased gradually with the increase in the concentrations of H2O2. The absorbance intensity and the concentration of H2O2 showed a linear relationship between 5-50 µM. The calibration curve for H2O2 detection was presented in Figure 4C. The linear regression equation is Y=0.01228X+0.20931 with the correlation coefficient R2 of 0.99264. The detection limit is estimated to be 0.96 µM in terms of the rule of three times standard deviation over the blank.

Figure 4 (A) Experimental phenomenon of different concentrations of H2O2; (B) Absorbance curve of different concentrations of H2O2; (C) Standard curve of H2O2 at different concentrations
4. Conclusions
In summary, a highly sensitive TMB colorimetric H₂O₂ biosensor was constructed based on the good catalytic activity of H-rGO-Pt@Pd NCs for H₂O₂ and the color rendering of TMB. In the range of 5-50 µM, the absorbance intensity is linearly related to the concentration of H₂O₂, the regression equation was Y=0.01228X+0.20931 with a correlation coefficient of 0.99264. The H-rGO-Pt@Pd NCs has good peroxidase-like activities by the synergistic effect of Pt@Pd NPs, rGO and Hemin, which can greatly improve the sensitivity and stability of the non-enzymatic H₂O₂ biosensors.

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