Gold foil-based biosensor for the determination of hydrogen peroxide

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***Abstract*—Hydrogen peroxide (H_2O_2) plays a critical role in the regulation of multifarious physiological processes. We developed a sensor containing a mercaptopropionic acid (MPA) monolayer covalently immobilized with Horseradish peroxidase (HRP) enzyme for the electrochemical detection of hydrogen peroxide (H_2O_2). A gold foil substrate was chemically treated with nitric acid and were used as working electrode. Platinum wire and Ag-Ag/Cl were used as counter and reference electrodes, respectively. The acid treated gold electrode with the immobilized enzyme shown to have improved catalytic activity in the reduction of H_2O_2. The steady-state current response increases linearly with H_2O_2 concentration from 10 µM to 9 mM with a low detection limit of 60 µM and showed a sensitivity of 0.4 mA/ mM cm^2. This electrochemical sensor is demonstrated to be highly selective and sensitive in the presence of interfering analytes. The improved activity and simple preparation method of the electrode makes the MPA-HRP modified gold electrode promising for being developed as an attractive robust material for electrochemical H_2O_2 sensing.**

I. Introduction

A simple, accurate and inexpensive determination of hydrogen peroxide (H_2O_2) concentration is an active area of research due to potential biological, industrial, and environmental applications [1, 2]. Biologically, H_2O_2 plays a significant role as a signaling and regulating molecule within cells and between cells [3, 4]. In addition, the concentration of H_2O_2 is a key biological parameter because H_2O_2 is a reactive oxygen species (ROS), which is a product of metabolism in the human body [5] and results in oxidative stress [6,7]. High levels of oxidative stress caused by ROS may damage DNA and contribute to cancer [8]. Quantitative analyses of H_2O_2 are indicative of oxidative stress and easily determined in the intact human organism [9]. Therefore, electrochemical sensors are utilized in the detection of various analytes owing to their scalable size and good temporal resolution [10]. Noble metals, such as platinum, gold and silver, as well as their alloys, show good catalytic activity towards H_2O_2 oxidation and reduction [11–13].

* This work was supported by Department of Defense USAMRMC (Award W81XWH-17-1-0452).

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To improve the electrochemical reduction of H_2O_2, many attempts were made based on using peroxidases to modify electrodes, which exhibited good specificity and sensitivity towards H_2O_2 [14]. Among peroxidases, HRP has been one of the most widely studied enzymes in the development of enzyme-based amperometric biosensors as a result of their availability and low cost. This enzyme contains heme as a prosthetic group, which is also the protein active site with the resting state of the heme-iron: Fe(II), and it can catalyze the H_2O_2 dependent one-electron oxidation of a great variety of substrates [15]. However, it is difficult for the electron transfer between HRP and electrode to occur, for the deep embedding of the HRP active site. So it is a challenge to develop specific immobilization methods and materials for achieving direct electron transfer. Nowadays several valuable immobilization strategies have been employed including absorption [16], layer-by-layer assembly [17], entrapment [18], and electropolymerization [19, 20].

With the development of scientific technologies, numerous materials are used as carriers for HRP, composing of polymer [18, 19], nanomaterial [20, 21], mesoporous material [22], and hybrid material [23]. But still, cross linking [24] technique makes the immobilization more reliable because of its covalent bonding. Moreover, porous electrodes have attracted much interest due to their good performances in numerous applications like reactors, fuel cells, batteries, sensors. An established preparation method is based on slow metal electrodeposition through solid templates and final removal of the latter, typically by etching or dissolution [25]. This method allows deposition of many materials including alloys and noble metals, as well as provides excellent control over shape, size and organization [26–30].

Here, in this paper we have developed a sensitive sensor for H_2O_2 using covalent immobilization of HRP over MPA monolayer modified acid treated gold electrode. We have adopted the strategy of creating more surface area over the electrode to increase enzyme loading. For this purpose, gold foil was chemically etched. This surface enabled an even distribution of HRP by reducing the pinhole defects. The electrode was characterized by cyclic voltammetry (CV) and chronoamperometry (CA).

II. Materials and Methods

A. Materials

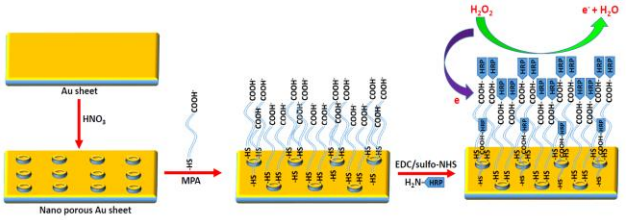
1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and N-hydroxysuccinimide (NHS) was bought from Pierce chemicals. Gold foil (99.99%), glucose, uric acid, ethanol,

ascorbic acid, acetaminophen, sodium hydroxide, MPA, potassium phosphate monobasic, HRP and hydrogen peroxide (H_2O_2) were acquired from Sigma-Aldrich. Phosphate buffer (PBS) pH 7.4 were prepared with 18.2 MW_cm Milli-Q water.

Cyclic voltammetry and amperometry were performed on a BASI-Epsilon electrochemical workstation. The three electrode system consisted of a platinum wire counter electrode, a Ag-Ag/Cl reference electrode and the gold foil (4 x 4 mm) as working electrode. All electrochemical experiments were carried out in a conventional electrochemical cell holding 25 ml of 100 mM PBS pH 7.4 at room temperature.

B. Fabrication of acid treated Au-MPA/HRP electrode

The gold foil was cut into 4 x 4 mm and cleaned with IPA for 10 minutes, dried with N_2. Before cleaning, 5 cm length titanium wire was soldered to one end of the foil electrode. The cleaned foil was incubated overnight in concentrated nitric acid to etch the foil. After incubation, the electrode was repeatedly cleaned with de-ionized (DI) water to remove acid residue and was stored in a desiccator. Before modification, the electrode was rinsed with ethanol for 5 minutes, dried with N_2. Immobilization of HRP over the MPA monolayer modified gold foil and its H_2O_2 sensing behavior are presented in Scheme 1.



Scheme 1. Electrode modification and sensing mechanism.

Briefly, the cleaned electrode was immersed a solution of MPA for 1 hour and subsquently washed with PBS to remove any unbound MPA. The carboxylic acid group of MPA was then activated by 0.8 mg EDC and 2.2 mg NHS along with 3 mg of HRP in 100 mM PBS (pH 7.4) for 2.5 hours. In presence of the EDC, the N-hydroxyl group of the NHS interacts with the carboxyl groups to form reactive sites, where the amino group of HRP displaces the succinimide group of NHS to form covalent bonding [31].

III. Result and Discussion

A. Voltammetric behavior of Au-MPA/HRP electrode

Figure 1 illustrates the voltammetric behavior of un-modified (bare) (curve, 1 a & b) and HRP modified (curve, c & d) gold foil in the presence (curve, b & d) and absence of 1 mM H_2O_2 (curve, a & c). A redox peak at 780 mV and -320 mV for the unmodified gold electrode indicated the metallic property of the electrode. In the presence of 1 mM H_2O_2, the redox current for bare electrode was slightly enhanced and two new peaks for H_2O_2 were observed at 130 and -330 mV respectively. This can be attributed to the presence of more active sites on the acid treated gold electrode surface. After modifying the electrode with MPA-HRP a new peak appeared at 380 mV, which confirms the presence of the enzyme on the acid treated gold electrode surface. When the same electrode was measured in the presence of 1 mM H_2O_2, a prominent redox behavior was observed. The peaks for the acid treated electrode at 780 and -320 mV increased and a new enhanced H_2O_2 redox peaks at 510 and -780 mV was observed. Clearly, the H_2O_2 reduction current has increased nearly five times in the presence of enzyme from -1.04 x 10^{-4} (bare electrode) to -5.34 × 10^{-4}.

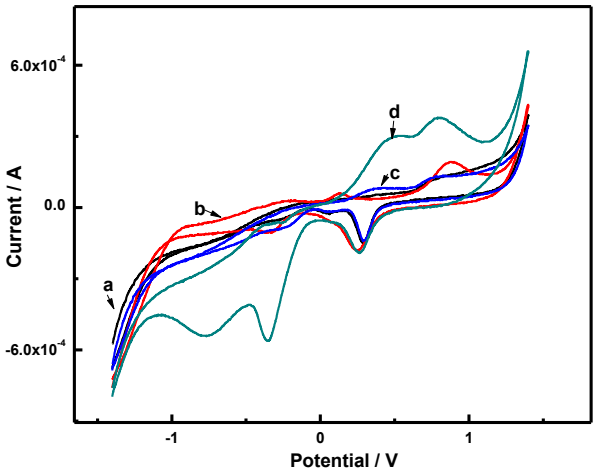


Figure 1. Voltammetric behavior of unmodified and MPA-HRP modified acid treated gold electrode in the presence and absence of 1 mM H_2O_2.

Further, the MPA-HRP modified acid treated gold electrode was also voltammetrically tested in the presence of lower H_2O_2 concentrations to show its biosensing applications within biological range 1, 25, 50 and 75 µM (Figure 2). A clear increment in the reduction current was observed when the H_2O_2 concentration was increased. The increase in the reduction peak currents were -2.79, -3.93, -4.37 and -4.70 × 10^{-4} for 1, 25, 50 and 75 µM of H_2O_2, respectively. This behavior show that the sensor can also work at low concentration regions, which can monitor the slightest variation of H_2O_2 concentration in biological fluid.

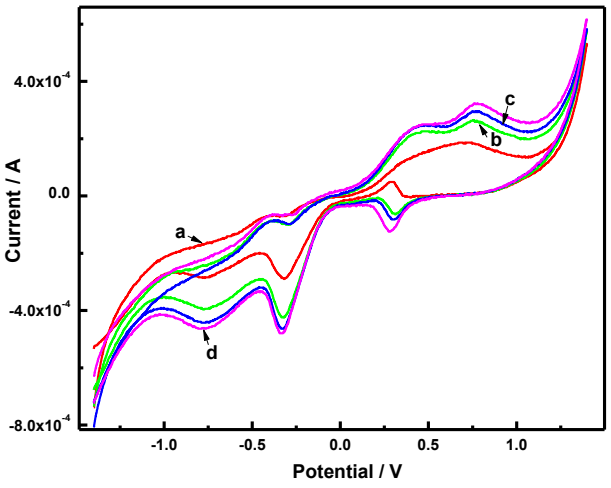


Figure 2. Voltammetric behavior of MPA-HRP modified acid treated gold electrode in the presence of 1, 25, 50 and 75 µM H_2O_2.

B. *Voltammetric behavior of Au-MPA/HRP electrode*

An amperometric technique was used to obtain calibration plot for H_2O_2. Figure 3 shows the amperometric behavior of MPA-HRP modified acid treated gold electrode in the successive addition of different concentrations of H_2O_2 (10 µM – 9.3 mM) at the applied potential of -760 mV. As demonstrated, each addition of H_2O_2 to the PBS (pH 7.4) solution, a sharp and well-defined cathodic peak current increased linearly and reached a steady state current. The response time of the sensor was slow at higher concentration regions. A calibration plot was plotted in the cathodic peak current vs. concentration of H_2O_2 as depicted in Fig. 3B. As a result, two linear ranges were observed. From the calibration plot, the limit of detection (LOD) and sensitivity was calculated to be 60 µM and 0.4 mA/mM cm^2, respectively. These two linear ranges phenomenon was also reported previously [32]. This may be due to the rapid formation of reduction products of H_2O_2 formed on the MPA-HRP modified acid treated gold electrode. Briefly, when the H_2O_2 concentration is high, a huge amount of reduction products was formed on the surface of the modified electrode, which prevented the adsorbing and reduction of H_2O_2. However, the obtained results revealed that the MPA-HRP modified acid treated gold electrode showed an improved analytical performance such as wide linear response range, very low LOD and good sensitivity and processes an excellent electrocatalytic ability for the quantitative determination of H_2O_2.

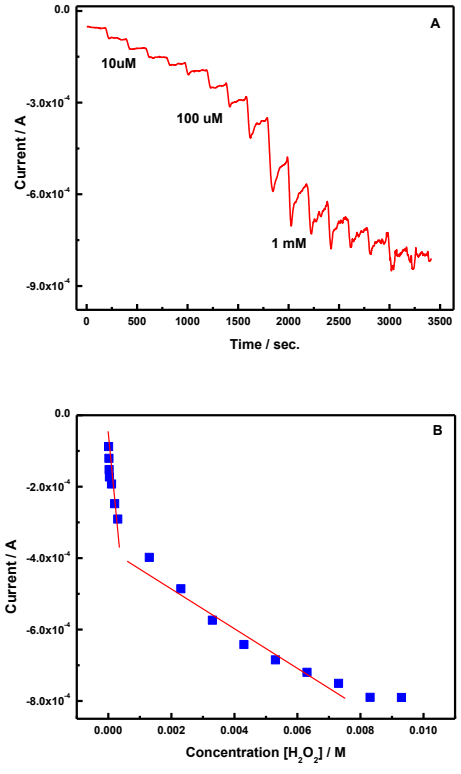


Figure 3. Amperometric response of MPA-HRP modified electrode and its (A) response upon the addition of different concentrations of H_2O_2 and (B) calibration curve.

C. *Selectivity*

To examine the selectivity of the MPA-HRP modified electrode, interference test was conducted (Figure 4). Four possible interfering substances (1 mM each) were used to evaluate the selectivity of the MPA-HRP electrode: glucose, uric acid, ascorbic acid and acetaminophen, which are other commonly found electroactive compounds [33, 34]. The current ratios were calculated by reading the current of the enzyme electrode in the assay solution containing 1 mM H_2O_2 and different interfering substance. The degree of interference from the substance can be judged from the current ratios. A big current change was observed upon the addition of 1 mM H_2O_2, while no significant increase in the cathodic current was observed after the addition of the interferences. The excellent selectivity may due to low operating potential (-760 mV) of detection. This result suggests that the MPA-HRP modified acid treated gold electrode has good selectivity towards H_2O_2.

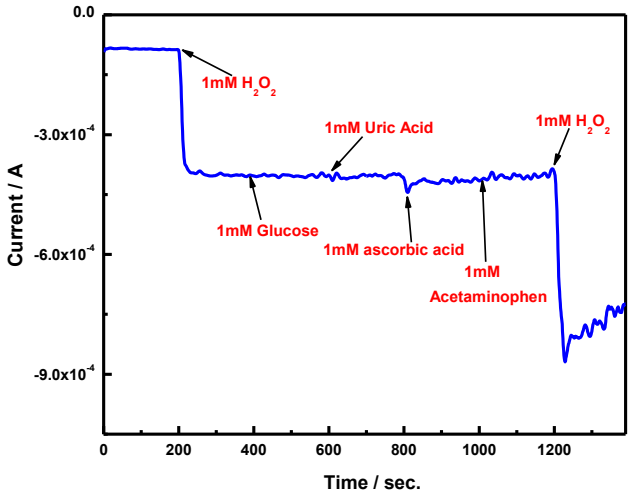


Figure 4. Amperometric performance of MPA-HRP modified acid treated gold electrode in the presence of different interferences.

IV. Conclusion

In this study, we have developed an enzymatic biosensor for the determination of hydrogen peroxide using electrochemical methods. An acid treated Au electrode was functionalized with HRP and tested in the presence and absence of hydrogen peroxide. The system showed a linear dynamic range from 60 µM to 9 mM. It also showed a higher sensitivity of 0.4 mA/mM cm^2. The presence of potential interference species such as glucose, ascorbic acid, uric acid and acetaminophen did not affect the performance of the sensor. The overall results indicated that the MPA-HRP modified acid treated gold electrode had excellent electrocatalytic activity toward H_2O_2 reduction and can be used to fabricate an enzymatic sensor with high sensitivity and selectivity.

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