SAGE-Hindawi Access to Research
International Journal of Electrochemistry
Volume 2011, Article ID 603257, 4 pages
doi:10.4061/2011/603257

Research Article

Functionalized Palladium Nanoparticles for Hydrogen Peroxide Biosensor

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Received 30 March 2011; Accepted 30 April 2011

Academic Editor: Farnoush Faridbod

We present a comparison between two biosensors for hydrogen peroxide (H_2O_2) detection. The first biosensor was developed by the immobilization of Horseradish Peroxidase (HRP) enzyme on thiol-modified gold electrode. The second biosensor was developed by the immobilization of cysteamine functionalizing palladium nanoparticles on modified gold surface. The amino groups can be activated with glutaraldehyde for horseradish peroxidase immobilization. The detection of hydrogen peroxide was successfully observed in PBS for both biosensors using the cyclic voltammetry and the chronoamperometry techniques. The results show that the limit detection depends on the large surface-to-volume ratio attained with palladium nanoparticles. The second biosensor presents a better detection limit of 7.5 μM in comparison with the first one which is equal to 75 μM.

1. Introduction

The scope of H_2O_2 is very broad in our days, it affects many areas like chemistry [1], food industries [2], clinical applications, and environmental chemistry [3]. Many determinations methods of hydrogen peroxide have been developed using titrimetry [4, 5], spectrophotometry [4, 6], chemiluminescence [4, 7], and electrochemical [2, 4, 8–10]. The first three techniques reveal inaccuracy and are complex and expensive [10]. Besides, the electrochemical methods are very selective, sensitive, and illustrate low detection limits for hydrogen peroxide [2, 8, 11].

Between numerous enzymes, horseradish peroxidase (HRP) has commonly been chosen to detect H_2O_2 due to the easy availability in high purity and low cost [12–14]. Nanoparticles have received considerable attention in these last years. Nanoparticles can bring many advantages when they are immobilized on an electrode surface. They have a large surface-to-volume ratio which contributes to the probability of electrocatalytic activity [9, 15, 16]. Palladium nanoparticles with small size (1 nm) were used due to their higher electron conductivity. In this present work, two biosensors were developed for hydrogen peroxide detection. The first biosensor was developed by the immobilization of Horseradish Peroxidase (HRP) enzyme on thiol modified gold electrode. The second biosensor was developed by the immobilization of cysteamine functionalizing palladium nanoparticles on modified gold surface. The detection of hydrogen peroxide was successfully observed in PBS for both biosensors using the cyclic voltammetry and the chronoamperometry techniques. The results show that the limit detection depends on the large surface-to-volume ratio attained with small palladium nanoparticles.

2. Experimental Setup

2.1. Reagents. All chemicals were commercially available and used as received. The Palladium acetate was purchased from Strem Chemicals (USA). Horseradish peroxidase, n-dodecyl sulfide, 16-mercaptohexadecanoic acid (MHDA) 90%, cystaminedihydrochloride, 1-ethyl-3-(3-(dimethylamino)-propyl) carbodiimide (EDC), and N-hydroxy succinimide (NHS) were purchased from Sigma Aldrich (USA). The buffer solution used for all experiments was phosphate buffered saline (PBS) containing 140 mM NaCl, 2.7 mM

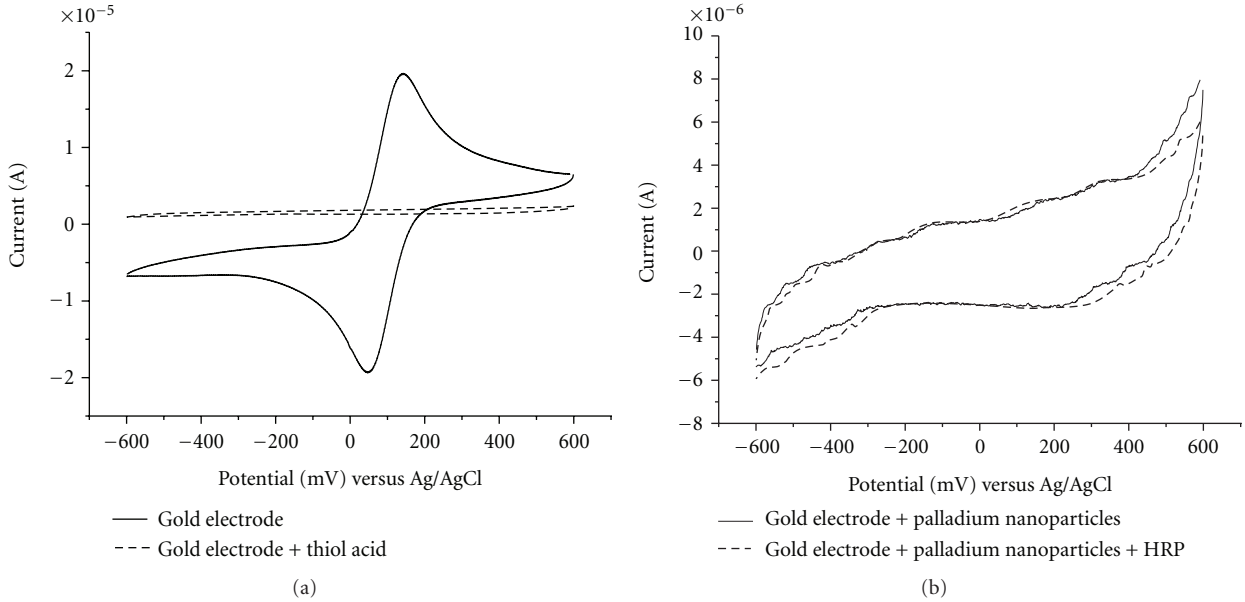

Figure 1: (a) Cyclic voltammogram of gold electrode and gold electrode with thiol acid. (b) Cyclic voltammogram of gold electrode with palladium nanoparticles and gold electrode with palladium nanoparticles with HRP.

KCl, 0.1 mM Na_2HPO_4, 1.8 mM KH_2PO_4, pH 7.0, and the redox couple $Fe(CN)_6^{3-}/Fe(CN)_6^{4-}$ at 5 mM concentration. All reagents were of analytical grade and ultrapure water (resistance, 18.2 MΩ·cm^{-1}) produced by a Millipore Milli-Q system was used throughout.

2.2. Instrumentation. Cyclic voltammetry and chronoamperometry measurement were performed at room temperature in a conventional voltammetric cell with a three electrode configuration using Autolab impedance analyzer (Ecochemie, The Netherlands). The gold electrode (0.16 cm^2) was used as working electrode, platinum (1 cm^2) and Ag/AgCl electrodes were used as counter and reference electrodes, respectively. All the electrochemical measurement were carried out in PBS at pH 7.0 with 5 mM $Fe(CN)_6^{3-}/Fe(CN)_6^{4-}$ and in Faraday cage.

2.3. Sensors Development. For the first biosensor, the gold electrode was immersed in an ethanol solution containing 1 mM of MHDA for 12 hours at room temperature. The treated electrode was then immersed in a solution of EDC (0.4 mM) and NHS (0.1 mM) for 1 h. A drop of HRP solution with a concentration of 100 μg/mL was deposited on the treated electrode for 1 hour. Then, a drop of a 1% BSA solution was added on the substrate for 30 min to block the free spaces between the enzyme and the SAM.

For the second biosensor, gold electrode was immersed in 1 mM of MHDA solution for 12 h, then activated with EDC and NHS for 1 h. After that, the electrode was immersed in a solution of Pd nanoparticles (1 nm size) functionalized previously with cysteamine dihydrochloride for 12 hours. The amine-thiol groups can be activated by immersing the substrate in PBS solution containing 5% (v/v) glutaraldehyde (GA) for 2 h. After rinsing with PBS, a solution of HRP (100 μg/mL) was added onto the surface for 1 hour to achieve the base reaction between the aldehyde group and the amino group of the enzyme. Then, a drop of a 1% BSA solution was added on the substrate for 1 hour to block the unspecific sites.

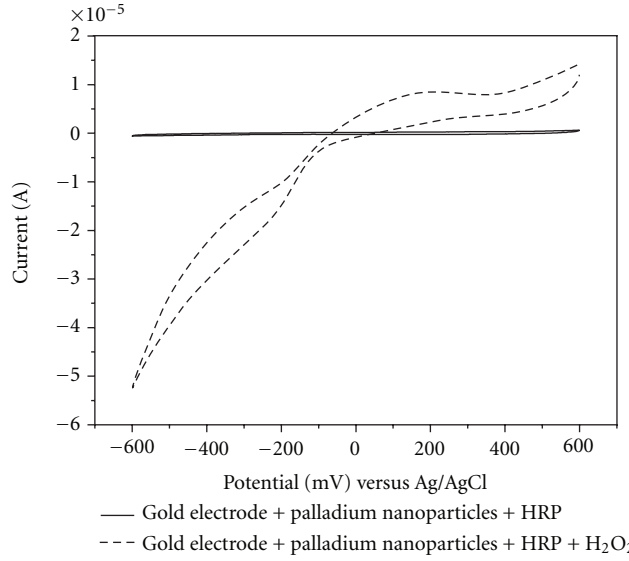

Figure 2: Cyclic voltammogram of gold electrode with palladium nanoparticles functionalized with HRP with 1,5 mM H_2O_2.

3. Results and Discussions

The synthesis and morphological characterization of Pd nanoparticles have been discussed elsewhere [17]. Cyclic voltammetry is a convenient methodology for studying

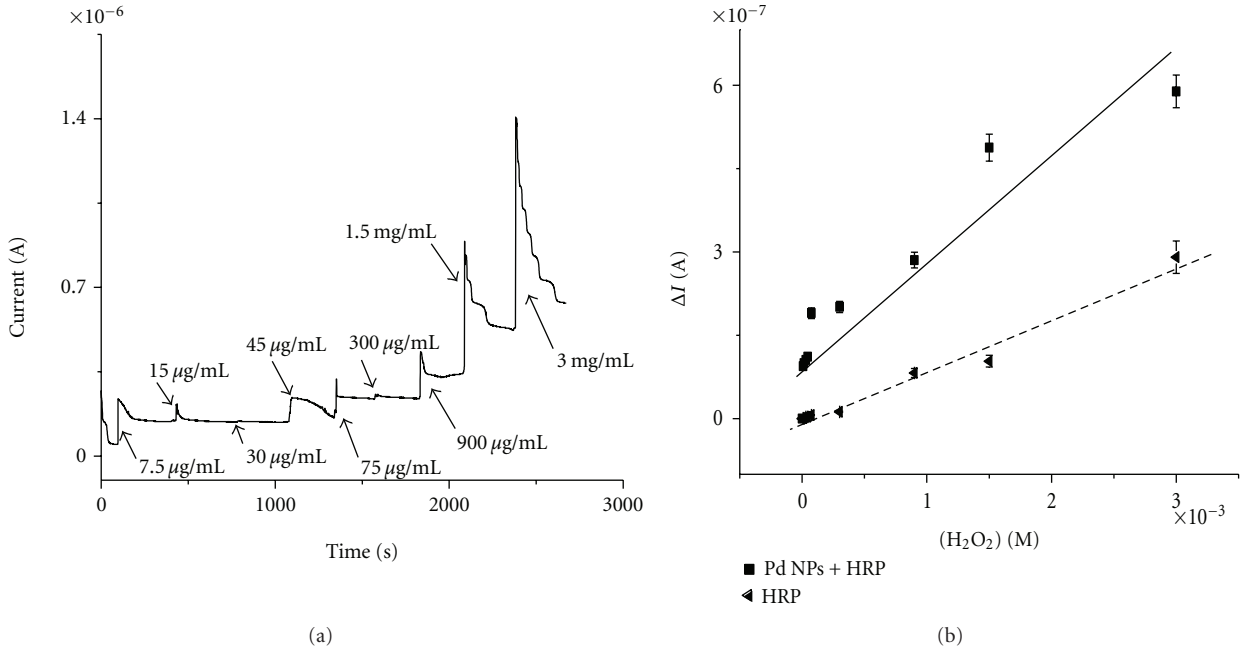

Figure 3: (a) Chronoamperometry curve of gold electrode with thiol acid and with Pd NPs and with HRP. (b) Calibration curve of gold electrode with thiol acid and Pd NPs and HRP (squares) and gold electrode with thiol acid and HRP and BSA (triangles) with different concentrations of H_2O_2.

the kinetics of an oxidation reduction reaction and the electrical properties of material in electrolyte interface. Figure 1(a) shows a typical cyclic voltammogram of the bare gold electrode, where a two peaks correspond to the ferrocyanide redox couples. After thiol deposition, the two current peaks disappeared showing the high insulating properties of the thiol monolayer. After the palladium nanoparticles deposition, the current decreases gradually due to the insulating properties of cysteamine covering the nanoparticles. The same behavior was obtained after HRP immobilization (Figure 1(b)). Figure 2 shows a cyclic voltammogram of the gold electrode functionalized with palladium nanoparticles and with HRP molecule before and after injection of H_2O_2 (1.5 mM). Upon the addition of the hydrogen peroxide to the electrochemical cell, the reduction peak (at −200 mV) appears, showing a typical electron transfer between the H_2O_2 and the HRP molecule [14]:

$$\begin{aligned} H_2O_2 + HRP_{red} &\longrightarrow HRP_{ox} + H_2O \\ HRP_{ox} + 2e^- &\longrightarrow HRP_{red} \end{aligned} \quad (1)$$

The chronoamperometry can be used to explore the current response of the biosensor in presence of the hydrogen peroxide. Figure 3(a) shows the chronoamperometry curve of functionalized electrode with palladium nanoparticles with HRP at a fixed potential −200 mV after H_2O_2 injections. The current increases with increasing H_2O_2 concentration. Figure 3(b) shows the current time curves of the two biosensors exposed to different concentration of hydrogen peroxide. The addition of the hydrogen peroxide to the buffer solution increases the steady state current. The curves were represented by a linear regression and a best sensitivity was obtained with palladium nanoparticles. A limit detection of 75 μM and 7.5 μM H_2O_2 was obtained with a good reproductibility for the first and the second biosensors, respectively. This difference in sensitivity and limit detection were due to the large surface to volume ratio given by the palladium nanoparticles. Moreover, the small size of nanoparticles (1 nm) increases the electron confinement in palladium which induces a higher electric conductivity, thus, an easier electron exchange.

4. Conclusion

In this paper, we present a comparison between two new biosensors for the detection of hydrogen peroxide (H_2O_2). The first biosensor was developed by the immobilization of Horseradish Peroxidase (HRP) enzyme on thiol modified gold electrode. The second biosensor was developed by the immobilization of cysteamine functionalizing palladium nanoparticles on modified gold surface. The amino-groups can be activated with glutaraldehyde for horseradish peroxidase immobilization. The detection of hydrogen peroxide was successfully observed in PBS for both biosensors using the cyclic voltammetry and the chronoamperometry techniques. The results show that the biosensor response depends on the conductivity and the large surface-to-volume ratio attained with palladium nanoparticles.

Acknowledgments

The authors thank Pr. Sherine Obare (University of Michigan, USA) and Pr. N. Jaffrezic-Renault (UCB-Lyon1, France) for their collaboration.

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