A biosensor by using Modified glassy carbon electrode With HRP Enzyme and Zro₂ Nanoparticles for Detect of H₂O₂

Mohamad Fazilati

Department of Biology, Payame Noor University, I.R. of IRAN

ABSTRACT

Design a biosensor by using Modified glassy carbon electrode With HRP Enzyme and Zro₂ Nanoparticles for Detect of H₂O₂ was studied. The heme group of HRP Enzyme played an important role in reduction (Fe²⁺) an oxidation (Fe³⁺) state in our electrochemical experiments. The phase characterization of ZrO₂ nanoparticles was performed by X-ray diffraction (XRD) using a D/Max-RA diffractometer with CuKα radiation. The absorbance properties of prepared nanoparticles were measured and recorded by using a TU-1901 double-beam UV-visible spectrophotometer. The morphologies and particle sizes of the samples were characterized by JEM-200CX transmission electron microscopy (TEM). The formal potential (E⁰) for the HRP redox reaction on the HRP / ZrO₂ NPs/ GCE was equal to +0.49. This experiment has introduced a new biosensor for determination of H₂O₂ in the range of 50 to 400 µM.

INTRODUCTION

Nanomaterials are chemical substances or materials that are manufactured and used at a very small scale (down to 10,000 times smaller than the diameter of a human hair) [1-4]. Nanomaterials are developed to exhibit novel characteristics (such as increased strength, chemical reactivity or conductivity) compared to the same material without nanoscale features [5-6]. Nanomaterials have the potential to improve the quality of life; Metal nanoparticles (Nps) can be used to enhance the amount of immobilized biomolecules in construction of a sensor [7]. Metal nanoparticles have been used to catalyze biochemical reactions and this capability can be usefully employed in biosensor design. Nanotechnology is having a profound effect on the development of new biosensors. Biosensors commonly comprise a biological recognition molecule immobilised onto the surface of a signal transducer to give a solid state analytical device [8-9]. The reaction between the biorecognition molecule and the analyte is a heterogeneous reaction and therefore the design of the biosensing interface is all important in determining the final performance of the biosensor [10-11]. Advances in nanofabrication of biosensing interfaces are one of the two major areas where nanotechnology has dramatically impacted on biosensor research in the last few years. The enzyme horseradish peroxidase (HRP), found in horseradish, is used extensively in biochemistry applications primarily for its ability to amplify a weak signal and increase detectability of a target molecule. Horseradish peroxidase is a 44,173.9-dalton glycoprotein with 6 lysine residues which can be conjugated to a labeled molecule. It produces a coloured, fluorimetric, or luminescent derivative of the labeled molecule when incubated with a proper substrate, allowing it to be detected and quantified [12]. HRP is often used in conjugates (molecules that have been joined genetically or chemically) to determine the presence of a molecular target [13]. The 3D structure of HRP was shown in figure 1. The heme group is clear in center of HRP structure with yellow color; this heme group played an important role in reduction (Fe²⁺) an oxidation (Fe³⁺) state in our electrochemical experiments [14].
Here we designed a biosensor by using Modified glassy carbon electrode With HRP Enzyme and ZrO$_2$ Nanoparticles for Detect of H$_2$O$_2$.

MATERIALS AND METHODS

2.1. Materials
HRP and Zirconyl chloride octahydrate purchased from Sigma-Aldrich. Other Reagents purchased from Merck. The supporting electrolyte used for all experiments was 0.1 M pH 7 phosphate buffer solution (PBS), which prepared by using 0.1 M Na$_2$HPO$_4$ and NaH$_2$PO$_4$ solutions. All the reagents used were of analytical grade and all aqueous solutions were prepared using doubly distilled water generated by a Barnstead water system.

2.2. Apparatus
Cyclic voltammetry (CV) and square wave voltammetry were performed using an Autolab potentiostat PGSTAT 302 (Eco Chemie, Utrecht, The Netherlands) driven by the General purpose Electrochemical systems data processing software (GPES, software version 4.9, Eco Chemie). A conventional three-electrode cell was employed throughout the experiments, with bare or ZrO$_2$ nanoparticles modified glassy carbon electrode (3.0mm diameter) as a working electrode, a Ag/AgCl as a reference electrode, and a platinum electrode as a counter electrode. The phase characterization of ZrO$_2$ nanoparticles was performed by means of X-ray diffraction (XRD) using a D/Max-RA diffractometer with CuKα radiation. The absorbance properties of prepared nanoparticles were measured and recorded by using a TU-1901 double-beam UV-visible spectrophotometer. The morphologies and particle sizes of the samples were characterized by JEM-200CX transmission electron microscopy (TEM) working at 200 kV.

2.3. Preparation of ZrO$_2$ nanoparticles
The ZrO$_2$ nanoparticles were prepared according to the literature. Initially, 2.58 g ZrOCl$_2$·8H$_2$O and 4.80 g urea were dissolved in 20.0 mL CH$_3$OH under stirring to form a colorless solution. The solution was transferred to a 20-mL Teflon-lined stainless steel autoclave, which was heated to 200 °C and maintained at that temperature for 20 h. The obtained white product was post-treated with sulphuric acid solution (0.16 mmol), and then calcined at 645 °C.

2.4. Preparation of unmodified glassy carbon electrode
The most commonly used carbon-based electrode in the analytical laboratory is glassy carbon (GC). It is made by pyrolyzing (Pyrolysis is the decomposition of organic compounds by heating to high temperatures in the absence of oxygen) a carbon polymer, under carefully controlled conditions, to a high temperature like 2000°C [15]. An intertwining ribbon-like material results with retention of high conductivity, hardness, and inertness. Glassy carbon electrode (GCE, dia. 3mm) was polished with 1 µm and 0.05 µm alumina slurries sequentially and then rinsed with distilled water. After that, the electrode was sonicated in deionized water and finally dried under ambient conditions.

2.5. Preparation of modified GCE with ZrO$_2$ Nanoparticles and HRP
To prepare the modified GCE with ZrO$_2$ Nanoparticles and HRP, after immobilized ZrO$_2$ Nanoparticles on GCE surface, The ZrO$_2$ Nanoparticles / GCE was placed into a fresh PBS including 12mg mL$^{-1}$ HRP (pH 7.0, 3 to 5°C) for 8 hour. At the end, the modified electrode was washed with deionized water and placed in PBS (PH 7.0) at a refrigerator (3 to 5°C), before being employed in the electrochemical measurements as the working electrode.
RESULTS AND DISCUSSION

3.1. Microscopic characterization
The average diameter of the Synthesized ZrO$_2$ nanoparticle is about 20 nm, and has a very narrow particle distribution. The scale bare for this image also was 20 nm. This statement illustrated in figure 2. This Figure Show a TEM picture of the ZrO$_2$ nanoparticles.

![TEM images of ZrO$_2$ NPs, with diameter about 20 nm](image)

3.2. X-Ray diffraction of ZrO$_2$ nanoparticles
The XRD pattern (figure 3) for ZrO$_2$ nanoparticles, the diffraction peaks are absorbed at 20 values. The prominent peaks have been utilized to estimate the grain size of sample with the help of Scherrer equation [16] $D = \frac{K\lambda}{\beta \cos \theta}$ where $K$ is constant(0.9), $\lambda$ is the wavelength($\lambda = 1.5418$ Å) (Cu K$_{\alpha}$), $\beta$ is the full width at the half-maximum of the line and $\theta$ is the diffraction angle. The grain size estimated using the relative intensity peak for ZrO$_2$ nanoparticles was found to be 20 nm and increase in sharpness of XRD peaks indicates that particles are in crystalline nature. All different peaks in figure 3 related to ZrO$_2$ nanoparticles and matched to Joint Committee for Powder Diffraction Studies (JCPDS).

![XRD pattern for ZrO$_2$ nanoparticles](image)
3.3. UV–visible absorption spectra for ZrO$_2$ nanoparticles

The most dramatic property of nanoparticles is the size evolution of the optical absorption spectra. Hence UV-visible absorption spectroscopy is an efficient technique to monitor the optical properties of quantum-sized particles. The UV–visible absorption spectra of ZrO$_2$ nanoparticles was shown in Figure 4; although the wavelength of our spectrometer is limited by the light source, the absorption band of the ZrO$_2$ nanoparticles have been shows a blue shift due to the quantum confinement in sample compare with bulk ZrO$_2$ particles. This optical phenomenon indicates that these nanoparticles show quantum size effect [17].

![UV–visible spectra for ZrO$_2$ Nps](image)

Figure 4. UV-Vis absorption spectra for ZrO$_2$ nanoparticles.

![Cyclic voltammograms](image)

Figure 5. Cyclic voltammograms, using (a) the ZrO$_2$ NPs/ GCE in 0.1 M phosphate buffer and (b HRP / ZrO$_2$ NPs/ GCE in 0.1 M phosphate buffer (scan rate: 100 mV/s).

3.4. Direct voltammetric behavior of the HRP / ZrO$_2$ NPs/ GCE electrode

The integrity of the immobilized HRP construction and its ability to exchange electrons with the nanometerscale ZrO$_2$ particles surfaces were assessed by voltammetry. A macroscopic electrode was required to attain a large enough HRP sample to yield detectable direct oxidation and reduction currents. The comparative CVs for the ZrO$_2$
NPs/ GCE and HRP / ZrO$_2$ NPs/ GCE in 0.1 M PBS (pH 7.0) was obtained. These voltammograms are demonstrated in Figure. 5 (a,b). From this Figure, it was noticed that there were no voltammetric response on ZrO$_2$ NPs/ GCE (Fig. 5a), which, Fig. 5(b) depicts a well-defined pair of oxidation–reduction (redox) peaks, observed on the HRP / ZrO$_2$ NPs/ GCE at 100 mV s$^{-1}$ scan rate value. The HRP / ZrO$_2$ NPs/ GCE presented the reductive peak potential at +0.42 V and the corresponding oxidative peak potential at +0.56 V (at 100 mV s$^{-1}$), illustrating the adsorbed HRP on the nanometer-scale ZrO$_2$ particle surfaces. The difference of anodic and cathodic peak potential values was $\Delta E = -0.14$ V. These redox peaks were attributed to the redox reaction of the HRP electroactive center. The formal potential ($E^0$) for the HRP redox reaction on the HRP / ZrO$_2$ NPs/ GCE was +0.49 with respect to the reference electrode.
The collected voltammograms in Figure 6 (a) substantiated this statement that the nanometer-scale ZrO$_2$ particles could play a key role in the observation of the HRP CV response. On the grounds that the surface-to-volume ratio increases with the size decrease and because of the fact that the enzyme size is comparable with the nanometer-scale constant ($k_s$) was estimated according to the following equation [19-20]:

$$\frac{\log ks = \log(1-a) + (1-a) \log \alpha - \log \left( \frac{nFR}{nF + nF_1} \right)}{2.3RT}$$

Here, $n$ is the number of transferred electrons at the rate of determining reaction and $R$, $T$ and $F$ symbols having their conventional meanings. The average heterogeneous transfer rate constant ($ks$) value was calculated about, 1.81 $s^{-1}$.

3.5. Electro catalytic reduction of H$_2$O$_2$ on the HRP / ZrO$_2$ NPs/ GCE

Upon addition of H$_2$O$_2$ to 0.1M pH 7.0 PBS, the cyclic voltammogram of the HRP / ZrO$_2$ NPs/ GCE electrode for the direct electron transfer of HRP changed dramatically with an increase of reduction peak current and a decrease of oxidation peak current (Fig. 7a), while the change of cyclic voltammogram of bare or ZrO$_2$ NPs/ GCE was negligible (not shown), displaying an obvious electrocatalytic behavior of the HRP to the reduction of H$_2$O$_2$. The decreases of the oxidative peak current together occurred with the increases of the reductive HRP / ZrO$_2$ NPs/ GCE. The electro-catalytic process could be expressed as follows:

Figure 6. (a) CVs of HRP / ZrO$_2$ NPs/ GCE electrode in PBS at various scan rates, from inner to outer: 25, 50, 75, 100, 125, 150, 175, 200 and 225 mV s$^{-1}$, the relationship between the peak currents (ipa, ipc) vs., (b) the sweep rates and (c) the square root of sweep rates.
The calibration curve (Figure 7b) shows the linear dependence of the cathodic peak current on the $\text{H}_2\text{O}_2$ concentration in the range of 50 to 400 $\mu$M. In Figure 7b, at higher concentration of $\text{H}_2\text{O}_2$, the cathodic peak current decreased and remains constant. Upon addition of an aliquot of $\text{H}_2\text{O}_2$ to the buffer solution, the reduction current increased steeply to reach a stable value (Fig 7b). This implies electrocatalytic property of electrode. Thus, this experiment has introduced a new biosensor for the sensitive determination of $\text{H}_2\text{O}_2$ in solution.

Figure 7. (a) Cyclic voltammograms obtained at an HRP / ZrO$_2$ NPs/ GCE in 0.1M PBS (pH 7.0) for different concentrations of $\text{H}_2\text{O}_2$ and (b) the relationship between cathodic peak current of HRP and different concentrations of $\text{H}_2\text{O}_2$ (scan rate: 100 mVs$^{-1}$).

3.6. Stability of designed $\text{H}_2\text{O}_2$ biosensor
The stability of $\text{H}_2\text{O}_2$ biosensor has been checked by carrying out experiments at the regular interval of a week and it has been found that HRP / ZrO$_2$ Nps/ GCE electrode based electrochemical biosensor retains its 92% activity after
30 days. The loss in the activity of biosensor is not due to the denaturation of HRP but it is due to the poor adhesion of ZrO$_2$ Nanoparticles on the GCE.

CONCLUSION

Nanotechnology is having a profound effect on the development of new biosensors. In this paper, I have constructed for design a new biosensor for the sensitive determination of H$_2$O$_2$ in solution. The H$_2$O$_2$ biosensor had successfully demonstrated high stability, good linearity, better sensitivity, fast response time, and excellent selectivity towards H$_2$O$_2$.

REFERENCES