Research Article

Direct Electron Transfer of Cytochrome c on ZnO Nanoparticles Modified Carbon Paste Electrode

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The direct electrochemistry of cytochrome c (cyt c) immobilized on a modified carbon paste electrode (CPE) was described. The electrode was modified with ZnO nanoparticles. Direct electrochemistry of cytochrome c in this paste electrode was easily achieved, and a pair of well-defined quasireversible redox peaks of a heme Fe (III)/Fe(II) couple appeared with a formal potential ($E^0$) of $-0.303$ V (versus SCE) in pH 7.0 phosphate buffer solution (PBS). The fabricated modified bioelectrode showed good electrocatalytic ability for reduction of H2O2. The preparation process of the proposed biosensor was convenient, and the resulting biosensor showed high sensitivity, low detection limit, and good stability.

1. Introduction

Many fields of nanotechnology are based on physical and chemical interactions, involving nanoparticles of particular size and shape. Nanoparticles (NPs) played an important role in absorption/adsorption of (volatile) organic molecules and gases due to their large specific surface area and high surface energy [1]. Nanoscaled inorganic materials have received much more attention because of their high chemical inertness, nonswelling effect, high purity, and rigidity [2, 3]. In order to use the nanomaterials as sensors, one has to understand the peculiarities of both the synthesis and interaction mechanism during the sensing act. In recent years, the interest of researchers and engineers in gas and liquid-sensitive materials has grown substantially due to the progress in nanotechnology [4]. The nanomaterials as sensors, one has to understand the peculiarities of both the synthesis and interaction mechanism during the sensing act. In recent years, the interest of researchers and engineers in gas and liquid-sensitive materials has grown substantially due to the progress in nanotechnology [4]. This interest is primarily connected to the promising electronic properties of nanomaterials, their size dependence, and the possibility of controlling the material structure by using new experimental techniques. Electrochemical sensors provide unlimited opportunities for monitoring environments and making the world safer and cleaner [5, 6]. Such devices meet the environmental and security demands for monitoring electroactive pollutants or threat agents with high sensitivity, selectivity, and temporal resolution [7, 8]. Electrochemical detection is of particular significance in the development of aptasensors since it allows for high sensitivity and selectivity, simple instrumentation, as well as low endogenous background [6]. Zinc oxide (ZnO), a versatile semiconductor material, has been attracting attention because of the commercial demand for optoelectronic devices operating at blue and ultraviolet regions [4]. ZnO is a wurtzite-type semiconductor with band gap energy of 3.37 eV, and it has very large excitation binding energy (60 meV) at room temperature [9]. Recently, special attention has been devoted to the morphology, as ZnO can form different nanostructures [10]. Thermal stability, irradiation resistance, and flexibility to form different nanostructures are the advantages that expedite its potential wide applications in photodetectors [11–13], surface acoustic wave devices [14], ultraviolet nanolaser [15], varistors [16], solar cells [17], gas sensors [18], biosensors [19], ceramics [20, 21], field emission [22], and nanogenerator [23]. Cyt c plays an important role in the biological respiratory chain, whose function is to receive electrons from Cyt c reductase and deliver them to Cyt c oxidase. So the electrochemical study of Cyt c is very important [24]. Due to the difficulty of direct electron transfer between Cyt c and a bare electrode, some modified electrodes were used as a tool to investigate
the direct electrochemical property. The modifiers of these modified electrodes are organic [25] or inorganic [24, 25] compounds. They were found to promote the direct electron transfer of Cyt c at electrode surfaces. It is well accepted that Cyt c exhibits peroxidase activities, which can catalyze the reductive reaction of hydrogen peroxide. Determinations to H2O2 based on such catalytic interactions are widely reported [26]. Effective immobilization and maintenance to the bioactivity on a proper substrate are essential to get a stable and sensitive response signal. Many methods for protein immobilization are extensively investigated, such as physical and biophysical methods [27, 28].

2. Experimental

2.1. Materials. Cytochrome c was purchased from Sigma. The phosphate buffer solution (PBS) consisted of a potassium phosphate solution (KH2PO4 and K2HPO4 from Merck; 1 M total phosphate) at pH 7.0. All other chemicals were of analytical grade and were used without further purification. All solutions were made up with doubly distilled water.

2.2. Apparatus. Cyclic voltammetric experiments were performed with a model EA-201 Electro Analyzer (chemilink systems), equipped with a personal computer that was used for electrochemical measurement and treating of data. A conventional three-electrode cell was employed throughout the experiments, with bare or ZnO nanoparticles modified carbon paste electrode (4.0 mm diameter) as a working electrode, a saturated calomel electrode (SCE) as a reference electrode, and a platinum electrode as a counter electrode. The phase characterization was performed by means of X-ray diffraction (XRD) using a D/Max-RA diffractometer with CuKα radiation. Samples were measured and recorded using a TU-1901 double-beam UV-visible spectrophotometer and were dispersed in toluene solution. The morphologies and particle sizes of the samples were characterized by JEM-200CX transmission electron microscopy (TEM) working at 200 kV, and scanning electron microscopy (SEM) images were obtained with a ZIESS EM 902A scanning electron microscope.

2.3. Procedure

2.3.1. Synthesis of ZnO NPs. To prepare of ZnO NPs, in a typical experiment, a 0.45 M aqueous solution of zinc nitrate (Zn(NO3)2·4H2O) and 0.9 M aqueous solution of sodium hydroxide (NaOH) were prepared in distilled water. Then, the beaker containing NaOH solution was heated at the temperature of about 55°C. The Zn(NO3)2 solution was added dropwise (slowly for 1 h) to the above-heated solution under high-speed stirring. The beaker was sealed at this condition for 2 h. The precipitated ZnO NPs were cleaned with deionized water and ethanol then dried in air atmosphere at about 60°C.

2.3.2. Preparation of Carbon Paste Electrode. The carbon paste electrode was prepared by hand mixing of carbon powder (particle size 50 mm, density 20–30 g/100 mL) was mixed with the binder, silicone oil, in an agate mortar and homogenized using the pestle. The electrode consisted of a Teflon well, mounted at the end of a Teflon tube. The prepared paste was filled into the Teflon well. A copper wire fixed to a graphite rod and inserted into the Teflon tube served to establish electrical contact with the external circuit. The electrode surface of the working electrode was renewed mechanically by smoothing some paste off and then polishing on a piece of transparent paper before conducting each of the experiments. The experiments were performed in unstirred solutions.

2.3.3. Preparation of ZnO Nanoparticles Modified Carbon Paste Electrode. The ZnO-nanoparticle-modified carbon paste electrode was employed throughout the experiments, with bare or ZnO-nanoparticle-modified carbon paste electrode (4.0 mm diameter) as a working electrode, a saturated calomel electrode (SCE) as a reference electrode, and a platinum electrode as a counter electrode.

3. Results

3.1. Electron Microscopic Investigation of ZnO Nanoparticles. Morphology of the sample was investigated using SEM and TEM. Figures 1(a) and 1(b) of Figure 1 show the typical SEM and TEM images of the sample, respectively. The SEM image was captured in 500-nanometer size of ZnO nanoparticles, and the TEM image was captured in 90 nanometer size of ZnO nanoparticles.

3.2. X-Ray Diffraction of ZnO Nanoparticles. The X-ray diffraction data were recorded by using Cu Ka radiation (1.5406 Å). The intensity data were collected over a 2θ range of 20–80°. The average grain size of the samples was estimated with the help of Scherrer equation using the diffraction intensity of (101) peak. X-ray diffraction studies confirmed that the synthesized materials were ZnO with wurtzite phase, and all the diffraction peaks agreed with the reported JCPDS data, and no characteristic peaks were observed other than ZnO. The mean grain size (D) of the particles was determined from the XRD line broadening measurement using Scherrer equation [29]:

\[
D = \frac{0.89\lambda}{\beta \cos \theta},
\]

where λ is the wavelength (Cu Ka), β is the full width at half-maximum (FWHM) of the ZnO (101) line, and θ is the diffraction angle. A definite line broadening of the diffraction peaks is an indication that the synthesized materials are in nanometer range. The lattice parameters calculated were also in agreement with the reported values. The reaction
Figure 1: (a) SEM image and (b) TEM image of ZnO NPs.

Figure 2: XRD patterns of ZnO nanoparticles. (a) indicates standard XRD pattern, and (b) indicates sample XRD pattern.

3.3. UV-Visible Absorption Spectra for ZnO Nanoparticles. The UV-visible absorption spectra of ZnO nanoparticles are shown in Figure 3; although the wavelength of our spectrometer is limited by the light source, the absorption band of the ZnO nanoparticles shows a blue shift due to the quantum confinement of the excitons present in the sample compared with bulk ZnO particles. This optical phenomenon indicates that these nanoparticles show the quantum size effect [30].

3.4. Direct Voltammetric Behavior of the Cyt c/ZnO NPs/CPE Electrode. The integrity of the immobilized cytochrome c construction and its ability to exchange electrons with the nanometer-scale ZnO particles surfaces were assessed by voltammetry. A macroscopic electrode was required to attain a large enough cytochrome c sample to yield detectable direct oxidation and reduction currents. The comparative CVs for the ZnO NPs/CPE and Cyt c/Zn NPs/CPE electrodes in 0.1 M PBS (pH 7.0) were obtained. These voltammograms are demonstrated in Figures 4(a) and 4(b). From this figure, it was noticed that there was no voltammetric response on ZnO NPs/carbon paste electrode (Figure 4(a)) Figure 4(b) depicts a well-defined pair of oxidation-reduction (redox) peaks, observed on the Cyt c/Zn NPs carbon paste electrode at 100 mV/s scan rate value. The Cyt c/Zn NPs/carbon paste electrode presented the reductive peak potential at −0.325 V and the corresponding oxidative peak potential at −0.280 V (at 100 mV s⁻¹), illustrating the adsorbed cytochrome c on the nanometer-scale zinc oxide particle surfaces. The difference of anodic and cathodic peak potential values was ΔE = 0.045 V. These redox peaks were attributed to the redox reaction of the cytochrome c electroactive center. The formal potential (E⁰) for the cytochrome c redox reaction on the Cyt c/Zn NPs/carbon paste electrode was −0.303 V with respect to the reference electrode. The collected voltammograms in Figure 5(a) substantiated this statement that the nanometer-scale nickel oxide particles could play a key role in the observation of the cytochrome c CV response. On the grounds that the surface-to-volume ratio increases with the size decrease and because of the fact that the protein size is comparable with the nanometer-scale building blocks, these nanoparticles displayed a great effect on the electron exchange assistance between cytochrome c and carbon paste electrode. To further investigate the cytochrome c characteristics at the Cyt c/ZnO NPs/CPE electrode, the effect of scan rates on the cytochrome c voltammetric behavior was studied in detail. The baseline subtraction procedure for the cyclic voltammograms was obtained in accordance with the method reported by Bard and Faulkner [31]. The scan rate (ν) and the square root scan rate (ν¹/²) dependence of the heights and potentials of
the peaks are plotted in Figures 5(b) and 5(c). It can be seen that the redox peak currents increased linearly with the scan rate, the correlation coefficient was 0.9948 ($\text{ipc} = 0.0119\nu + 1.7389$) and 0.9937 ($\text{ipa} = -0.0168\nu - 1.6163$), respectively. This phenomenon suggested that the redox process was an adsorption controlled and the immobilized cytochrome c was stable. It can be seen that the redox peak currents increased more linearly with the $\nu$ in comparison to that of $\nu^{1/2}$.

However, there is clearly a systematic deviation from linearity in this data, that is, low scan rates are always on one side of the line and the high scan rate points are on the other. The anodic and cathodic peak potentials are linearly dependent on the logarithm of the scan rates ($\nu$) when $\nu > 1.0 \text{Vs}^{-1}$, which was in agreement with the Laviron theory, with slopes of $-2.3RT/\alpha nF$ and $2.3RT/(1 - \alpha)nF$ for the cathodic and the anodic peak, respectively [32]. So the charge-transfer coefficient ($\alpha$) was estimated as 0.55. Furthermore, the heterogeneous electron transfer rate constant ($ks$) was estimated according to the following equation [32, 33]:

$$
\log ks = \alpha \log(1 - \alpha) + (1 - \alpha) \log \alpha
- \log \frac{RT}{nF\nu} - \frac{\alpha(1 - \alpha)nF\Delta E_p}{2.3RT}.
$$

(2)

Here, $n$ is the number of transferred electrons at the rate of determining reaction and $R$, $T$, and $F$ symbols having their conventional meanings. $\Delta E_p$ is the peak potential separation. The $\Delta E_p$ was equal to 0.330, 0.450, and 0.512 V at 0.7, 1 and 2 V s$^{-1}$, respectively, giving an average heterogeneous transfer rate constant ($ks$) value of 0.64 s$^{-1}$.

### 3.5. Electrocatalytic Reduction of $\text{H}_2\text{O}_2$ on the Cyt $c$/ZnO NPs/CPE-Retained Electrode.

Upon addition of $\text{H}_2\text{O}_2$ to 0.1 M pH 7.0 PBS, the cyclic voltammogram of the Cyt $c$/ZnO NPs/CPE electrode for the direct electron transfer of cyt c changed dramatically with an increase of reduction peak current and a decrease of oxidation peak current (Figure 6(a)), while the change of cyclic voltammogram of bare or ZnO Nps/CPE was negligible (not shown), displaying an

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**Figure 4:** Cyclic voltammograms, using (a) the ZnO NPs/CPE in 0.1 M phosphate buffer and (b) Cyt c/Zn NPs/CPE in 0.1 M phosphate buffer (scan rate: 100 mV/s).

**Figure 5:** (a) CVs of Cyt c/ZnO NPs/CPE electrode in PBS at various scan rates, from inner to outer; 50, 100, 200, 300,400, 500, and 600 mV $\text{s}^{-1}$, the relationship between the peak currents (ipa, ipc) versus (b) the sweep rates and (c) the square root of sweep rates.
obvious electrocatalytic behavior of the cyt c to the reduction of \( \text{H}_2\text{O}_2 \). The decreases of the oxidative peak current together with the increases of the reductive Cyt c/ZnO NPs/CPE. The electrocatalytic process could be expressed as follows:

\[
\begin{align*}
\text{Cyt c Fe(III)} + \text{H}_2\text{O}_2 & \rightarrow \text{Compound I} + \text{H}_2\text{O} \\
\text{Compound I} + \text{H}_2\text{O}_2 & \rightarrow \text{cyt c Fe(III)} + \text{O}_2 + \text{H}_2\text{O} \\
\text{Cyt c Fe(III)} + \text{H}^+ + e^- & \rightarrow \text{cyt c Fe(II)} \text{ (at electrode)} \\
\text{Cyt c Fe(II)} + \text{O}_2 & \rightarrow \text{cyt c Fe(II)} - \text{O}_2 \text{ (fast)} \\
\text{Cyt c Fe(II)} - \text{O}_2 & \rightarrow 2\text{H}^+ + 2e^- \rightarrow \text{cyt c Fe(II)} + \text{H}_2\text{O}_2 \text{ (at electrode)}
\end{align*}
\]

Calibration curve (Figure 6(b)) shows the linear dependence of the cathodic peak current on the \( \text{H}_2\text{O}_2 \) concentration in the range of 30 to 510 \( \mu \text{M} \). In Figure 6(b), at higher concentration of \( \text{H}_2\text{O}_2 \), the cathodic peak current decreased and remained 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 (Figure 6(b)). 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.

4. Conclusions

Zinc oxide nanoparticles (ZnO NPs) were electrodeposited onto the surface of carbon paste electrode and assessed using SEM and TEM procedures. The direct electrochemistry of cytochrome c in the form of a cyt c/ZnO NPs/CPE electrode was assessed by cyclic voltammetry. These nanoparticles helped cyt c to have a favored orientation and reduce the effective electron transfer distance. Present data describes that the designed biosensor can be useful in the bioelectrochemical and medical studies.

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