

Direct Electron Transfer of Catalase and its Biosensing for H₂O₂

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Abstract. By immobilizing catalase on a functional nanocomposites containing ion-liquid, TiO₂ nano particles and carboxylic acid functionalized multi-walled carbon nanotubes, direct electron transfer of CAT was realized on the nanocomposites modified glassy carbon electrode. The electrochemical study showed that the formal potential and heterogeneous electron transfer constant of the prepared modified glassy carbon electrode were -0.163 V and 5.24 s⁻¹, respectively. The electrocatalysis study showed that the linear response range toward H₂O₂ and the apparent Michaelis–Menten constant were from 5 μM to 1000 μM and 54.3 μM, respectively.

Introduction

The direct electrochemistry of redox proteins and enzymes on the electrodes can not only obtain important information such as thermodynamics and dynamics, but also help to understand and understand the mechanism of their electron transfer in life and the construction of a biosensor without the need of mediator[1-6].

Catalase (CAT) is a dumbbell-shaped tetrameric heme containing enzyme (220-350kD)[7, 8]. Due to the large steric hindrance effect, the direct electron transfer between CAT and electrodes is usually difficult to observe.

Many nanomaterials have been applied to the electrochemical study of redox proteins. However, the strong hydrophobic effects of some nanomaterials may cause the conformational changes and inactivation of redox proteins. Therefore, it may be a new approach to realize direct electrochemistry of a redox protein by immobilizing it on a functional nanocomposites modified electrode [9-29].

In the present study, CAT was immobilized on a functional nanocomposites containing ion-liquid (IL), TiO₂ nano particles and carboxylic acid functionalized multi-walled carbon nano-tubes. Direct electron transfer of CAT was realized on a glassy carbon (GC) electrode and a novel biosensor for H₂O₂ was constructed.

Experimental

Reagents and Apparatus

CAT, Nafion (NF, 5% ethanol solution) and H₂O₂ (v/v 30%) were obtained from Sigma, Saint. Louis, MO, USA. TiO₂ nanoparticles and Multi-walled carbon nano-tubes (MWCNTs) were from Shenzhen Nanotech Port Co., Ltd, China. Other chemicals were of analytical grade. Double distilled water was also used in this research. An electrochemical workstation

(CHI650C, Shanghai CHI Instrument Co. Ltd., China) was applied for electrochemical measurements. A Pt wire, an Ag/AgCl and a GC electrode were applied as the counter, reference and working electrodes, respectively.

Fabrication of NF/CAT/IL-CMWCNTs-TiO₂/GC Electrode.

The Functionalization of MWCNTs was based on literature [27]. The modified electrode was prepared as following: at first, 2 μ l of the mixture of the prepared carboxylic acid functionalized multi-walled carbon nanotubes (CMWCNTs) (2 mg/ml) and TiO₂ nano particles (1 mg/ml) (ratio of volume: 1) was added to the working surface of a new prepared GC electrode. Then, 1 μ l IL was added on the electrode and dried for 4h at room temperature. Afterwards, 2 μ l CAT was added and dried for for 12 hours at 4 °C. Finally, 2 μ l NF was added for protection.

Results and Discussion

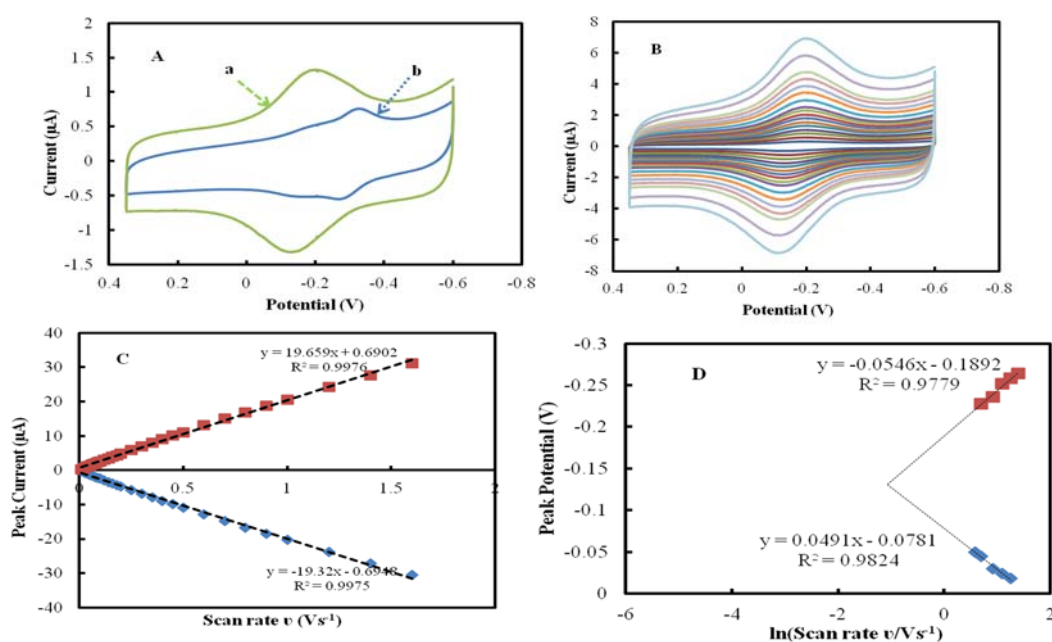


Figure 1. (A) CVs of (a) NF/CAT/IL-CMWCNTs-TiO₂/GC electrode and (b)NF/CAT/CMWCNTs-TiO₂/GC electrode, respectively, in 0.05 M PBS (pH 7.0) at a scan rate of 0.05 V/s. (B) CVs of NF/CAT/IL-CMWCNTs-TiO₂/GC electrode at various scan rates from 0.01 to 2.0 V/s. (C) Plot of peak current (I_p) versus scan rate v . (D) Plot of peak potential (E_p) versus logarithm of scan rate v .

The cyclic voltammograms (CVs) of: (a) NF/CAT/IL-CMWCNTs-TiO₂/ GC electrode; (b)NF/CAT/CMWCNTs-TiO₂/GC electrode are shown in Fig. 1A. The redox peaks of electrode (a), with a formal potential [$E^0=(E_{pa}+ E_{pc})/2$] of -0.163 V (versus Ag/AgCl), are obviously stronger than that of electrode (b). It seems that the IL may help to increase the redox peak currents significantly. Moreover, the direct electron transfer of CAT at a bare GC electrode was too slow to be observed (data not shown).

Scan rate effects on NF/CAT/IL-CMWCNTs-TiO₂/GC electrode are shown in Fig. 1B. The peak currents increased linearly with the increase of scan rate (v) (Fig. 1C), which means that the electroactive substance (CAT) has been immobilized on the electrode surface [30]. The

cathodic peak potential in the range from 1.0 to 2.0 V/s changes linearly with $\ln v$ (Fig. 1D). According to Eq. 1[31]:

$$E_p = E^{o'} + \frac{RT}{\alpha nF} - \frac{RT}{\alpha nF} \ln v \quad (1)$$

Where, R , T , α , n , and F are gas constant ($8.314 \text{ J}\cdot\text{K}^{-1}\cdot\text{mol}^{-1}$), temperature (298 K here), cathodic electron transfer coefficient, number of electrons, and Faraday constant ($96500 \text{ C}\cdot\text{mol}^{-1}$), respectively. Then, α and n were calculated to be 0.33 and 1, respectively [32].

By using the Eq.2[33], the electron transfer rate constant (k_s) was obtained to be 5.24 s^{-1} :

$$\ln k_s = a \ln(1-a) + (1-a) \ln a - \ln\left(\frac{RT}{nFv}\right) - a(1-a) \frac{nF\Delta E_p}{RT} \quad (2)$$

By using the slope of I_p versus v in Eq.3, the surface concentration (Γ) of CAT on the GC electrode was estimated to be $9.23 \times 10^{-11} \text{ mol/cm}^2$:

$$I_p = \frac{n^2 F^2 A \Gamma v}{4RT} \quad (3)$$

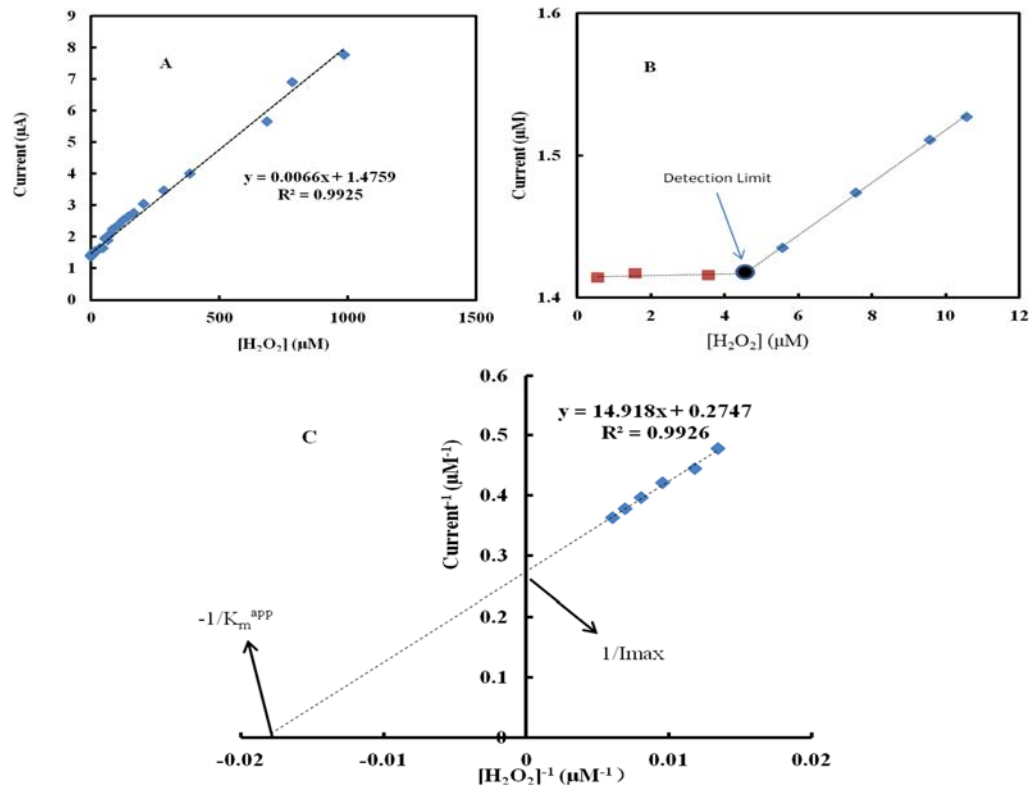


Figure 2. (A) The linear response range of the cathodic peak current of NF/CAT/IL-CMWCNTs-TiO₂/GC electrode toward H₂O₂. (B) Determination of the H₂O₂ detection limit for the modified GC electrode. (C) Lineweaver–Burk plot for K_{mapp} determination.

The cathodic peak current of increases with the increase of H₂O₂ concentration, and has a linear relationship with the concentration of H₂O₂ at 5.0 to 1000 μM (Fig. 2A). The detection limit is 5.0 μM (Fig. 2B). The apparent Michaelis–Menten constant (K_m^{app}) is 54.3 μM (Fig. 2C) from the electrochemical version of Lineweaver–Burk Eq.4 [5, 6, 30]:

$$\frac{1}{I_s} = \frac{1}{I_{max}} + \frac{K_m^{app}}{I_{max}c} \quad (4)$$

Where, c , I_s and I_{max} are H₂O₂ concentration in the bulk solution, steady-state current after the addition of H₂O₂ and maximum current measured under saturated substrate conditions, respectively.

Conclusions

Direct electron transfer CAT was realized on a functional IL-CMWCNTs-TiO₂ nanocomposites modified GC electrode. A novel biosensor for H₂O₂ was constructed by using the modified GC electrode.

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