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Direct Electrochemistry of Catalase on Single Wall Carbon Nanotubes Modified Glassy Carbon Electrode

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Abstract: Direct electrochemistry of catalase (Ct) has been studied on single wall carbon nanotubes (SWNTs) modified glassy carbon (GC) electrode. A pair of well-defined nearly reversible redox peaks is given at –0.48 V (*vs*. SCE) in 0.1 mol/L phosphate solution (pH 7.0). The peak current in cyclic voltammogram is proportional to the scan rate. The peak potential of catalase is shifted to more negative value when the pH increases. Catalase can adsorb on the SWNTs modified electrode.

Keywords: Carbon nanotubes, catalase, cyclic voltammetry, modified electrode.

Carbon nanotubes are very attractive materials, with the specific electronic, mechanical and chemical properties, since discovery in 1991[1]. There have been many applications of carbon nanotubes reported[2]. The electrochemical study of carbon nanotubes has been widely carried out, and this form of carbon shows better electrochemical behavior compared with other electrode materials[3]. Direct electrochemistry of proteins and enzymes, such as glucose oxidase[4], cytochrome c[5], myoglobin[6], and microperoxidase MP-11[7], on carbon nanotubes has been demonstrated, resulted from their high conductivity and peculiar properties.

Catalase (EC 1.11.1.6) is a redox enzyme, present in almost all aerobic organisms[8]. It catalyses the disproportionation of hydrogen peroxide into oxygen and water without the formation of free radicals, so it serves in part to protect cells from the toxic effects of hydrogen peroxide. Catalase contains four equal subunits, and each of them has a molecular weight of 57,000 Da., is equipped with a Fe (III)-protoporphyrin-IX. Electrochemistry of catalase on electrodes modified by films like bio-membranes, such as DDAB films[9], lipid films[10] and chitosan films[11] has been reported. Until now, there is no report on describing the electrochemistry of catalase on single wall carbon nanotubes. In this paper, the direct electron transfer of catalase on SWNTs modified GC electrode is demonstrated. A couple of well-defined redox peaks has been observed at –0.48 V (*vs*. SCE) in 0.1 mol/L phosphate solution (pH 7.0), while there is no electrochemical response of catalase on bare GC electrode. In cyclic voltammetric experiment the peak current grows linearly with the scan rate, and the peak potential of

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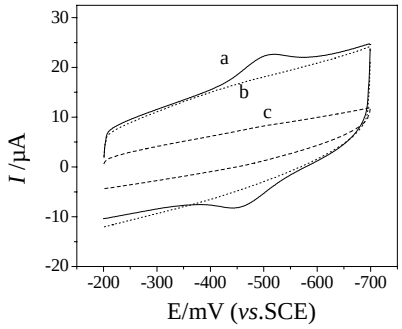
catalase is shifted to more negative value when the pH increases.

SWNTs were prepared by a direct current arc-discharge method with Y-Ni alloy as the catalyst[12], and purified using the method of oxidation in air[13]. Catalase (EC 1.11.1.6) was purchased from Sigma (from bovine liver, 2970 units/mg). Water was triply distilled from an all-quartz distiller. Other chemicals were of analytical grade. The SWNTs modified electrode was made by casting 15 μL SWNTs suspension in DMF (0.1 mg/mL) on the GC electrode polished with alumina, and dried under the infrared lamp, which was the same method as reported by the reference[14].

A BAS 100B electrochemical workstation (Bioanalytical Systems, Inc.) was used for cyclic voltammetry (CV) and for square wave voltammetry (SWV) experiments. The three-electrode cell employed a saturated calomel reference electrode (SCE), a platinum wire as counter electrode, and a glassy carbon (GC) as the working electrode (diameter 4mm). *Prior to* CV and SWV experiments the solutions were routinely deaerated by purging with nitrogen of high purity. Experiments were carried out at room temperature (25 ± 1 ^{o}C).

Figure 1 Cyclic voltammograms of catalase in 0.1 mol/L phosphate buffer (pH 7.0)

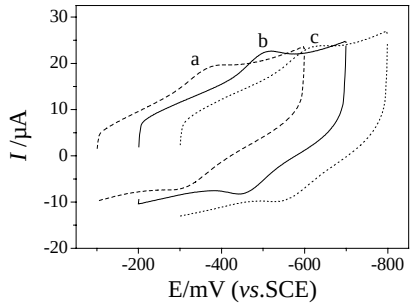


On the SWNTs modified GC electrode (a, b) and the bare GC electrode (c) immersing in 10 mg/mL catalase for 1.5 hours (a, c) and in the absence of catalase (b). Scan rate: 0.2 V/s.

Figure 1 shows the cyclic voltammograms (CV) of catalase on the SWNTs modified GC electrode and bare GC electrode. A pair of redox peaks was observed on the SWNTs modified GC electrode in 10 mg/mL catalase + 0.1 mol/L phosphate buffer solution (pH 7.0) (**Figure 1a**). In the absence of catalase, no peak was observed on the SWNTs modified electrode (**Figure 1b**). The peak currents of catalase increased with the immersing time, which shows catalase can adsorb on the SWNTs modified GC electrode. The effect of immersing time was examined by recording the CV and square wave voltammogram (SWV) at different immersing time. After about 1.5 hours the peak currents almost did not change. It suggests the saturated adsorption reached. In contrast, there were no redox peaks of catalase on bare glassy carbon electrode (**Figure 1c**). The formal potential ($E^{o'}$) of the catalase was –0.48 V (*vs*. SCE) (here $E^{o'} = (E_{pc} + E_{pa}) / 2$). These are consistent with the properties of the Fe^{III}/Fe^{II} redox couple[10,11].

In CV experiments, the peak currents were proportional to the scan rate in the range of 0.05 to 1.1 V/s. The peak width at half-height was 100 mV, and the separation of redox peaks was 44 mV at the scan rate of 0.2 V/s. These are in good agreement with the theory of ideal Nernstian reaction of adsorbed monolayer[15]. According to the theory[15] and by integration of the cyclic voltammetric peak, the catalase surface concentration obtained was 1.55×10^{-10} mol cm^{-2}, and the electron transfer number was determined to be 0.97. When the catalase saturated SWNTs modified GC electrode removed from the catalase solution, washed in triply distilled water, and then transferred in the 0.1 mol/L phosphate buffer solution, the redox peaks of catalase were still observed, and the electrochemical response was stable. In this paper, the following cyclic voltammetric experiments were all carried out under the saturated adsorption conditions.

Figure 2 The effects of pH on the cyclic voltammograms of catalase



On the SWNTs modified GC electrode in 10 mg/mL catalase + 0.1 mol/L phosphate buffer solution. The pH of buffer is: (a) 4.4, (b) 7.0 and (c) 9.2. Scan rate: 0.2 V/s.

Effects of pH on the redox reaction of catalase have been investigated. When the pH of buffer solution was increased, the $E^{o'}$ of catalase was shifted to more negative values. The CV curves are shown in **Figure 2**. The $E^{o'}$ changed linearly with the pH in the range of 4.4 to 9.2. The plot of $E^{o'}$ versus pH gave the slope of 52 mV/pH, which is close to the theory value 59 mV/pH at 25 ^{o}C for a reversible proton-coupled single-electron transfer. The results are consistent with the reference[10,11].

Based on the experiments described above, SWNTs can enable the realization of the direct electron transfer between catalase and GC electrode, which is in agreement with the facilitating electron transfer between proteins and the carbon nanotubes[4-7]. It can be attributed to that SWNTs can enhance the electrochemical signal with high conductivity and provide a biocompatible environment.

In conclusion, the direct electron transfer of catalase was realized successfully on the SWNTs modified GC electrode. The electrochemical signal of catalase was corresponding to the Fe^{III}/Fe^{II} redox couple. The SWNTs modified electrode provides an avenue to study the direct electrochemistry of proteins or enzymes, and can be used as biosensors.

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