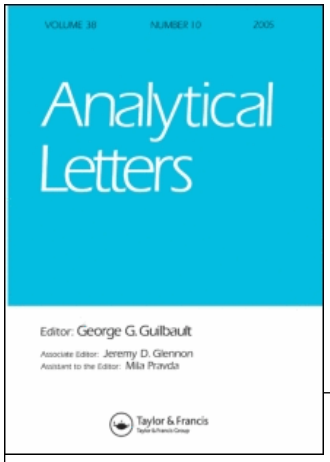

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Attachment of Nanoparticles to Pyrolytic Graphite Electrode and Its Application for the Direct Electrochemistry and Electrocatalytic Behavior of Catalase

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SENSORS

Attachment of Nanoparticles to Pyrolytic Graphite Electrode and Its Application for the Direct Electrochemistry and Electrocatalytic Behavior of Catalase

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Abstract: A highly hydrophilic, nontoxic, and conductive effect of colloidal gold nanoparticles (GNP) and multi-walled carbon nanotubes (MWCNT) on pyrolytic graphite electrode has been demonstrated. The direct electron transfer of catalase (CAT) was achieved based on the immobilization of MWCNT/CAT-GNP on a pyrolytic graphite electrode by a Nafion film. The immobilized catalase displayed a pair of well-defined and nearly reversible redox peaks in 0.1 M phosphate buffer solution (PBS) (pH 6.98). The dependence of $E^{o\prime}$ on solution pH indicated that the direct electron transfer reaction of catalase was a single-electron-transfer coupled with single-proton-transfer reaction process. The immobilized catalase maintained its biological activity, showing a surface controlled electrode process with an apparent heterogeneous electron transfer rate constant (k_s) of $1.387 \pm 0.1\ s^{-1}$ and charge-transfer coefficient (α) of 0.49, and displayed electrocatalytic activity in the electrocatalytic reduction of hydrogen peroxide. Therefore, the resulting modified electrode can be used as a biosensor for detecting hydrogen peroxide.

Keywords: Catalase; electrocatalysis; GNP; MWCNT; hydrogen peroxide

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INTRODUCTION

The combination of biological molecules and novel nanomaterials play an important role in developing new nanoscale devices for future biological, medical, and electronic applications (Willner 2002). Furthermore, reversible direct electron transfer between redox enzymes/proteins and an electrode surface is important in understanding the redox proteins, as well as in development of enzyme biosensors, biofuel cells, and biomedical devices (Heller 1992, 1999). However, one of the difficulties in electrochemical research is achieving direct electron transfer of enzymes and proteins on the surface of electrodes. Achieving the goal is important because direct electron transfer will offer an electrochemical basis for investigating the structure of enzymes and proteins and provide a model for the mechanistic study of redox process commonly observed in biological systems (Armstrong, Hill, and Walton 1988; Chapin and Bucke 1990). It is usually difficult for redox enzymes and proteins in solution phase to transfer electrons directly at bare solid electrodes due to various factors. Enzymes and proteins could be adsorbed on the electrode surface, resulting in large changes in its conformation and loss of its electrochemical activity and bioactivity. Furthermore, direct electrochemistry of enzymes and proteins at unmodified electrodes is usually prohibited because of shielding of the redox active sites deeply surrounded in the bodies (Li et al. 1997; Chaubey and Malhotra 2002; Shang et al. 2005) by the insulated protein shells, which results in the inaccessibility of the redox centers; the three-dimensional structures hinder interaction between electrons and electrodes and result in subsequent passivation of the electrode surfaces. Therefore, various types of films have been developed to immobilize proteins on the electrode surface to overcome these inhibitions, and the direct electrochemistry of proteins in the film phase has been realized (Rusling 2000; Armstrong and Wilson 2000).

It was reported that colloidal gold nanoparticles(GNP), are the most stable metal nanoparticles and have some unique and excellent properties, such as large specific surface area, high biocompatibility, good conductivity, and suitability for many surface immobilization mechanisms, so that they adsorb redox enzymes and proteins without loss of their biological activity. GNP are also used to immobilize redox enzymes and proteins on an electrode surface because they can act as tiny conduction centers and facilitate the transfer of electrons. Recently, GNP were successfully applied in the immobilization of myoglobin and used as an electron bridge between Mb and electrode, allowing reagentless biosensors for hydrogen peroxide and nitrite to be developed (Liu and Ju 2003; Shang and Oyama 2005). Carbon nanotubes also have attracted great interest owing to their unique structures and good electrical, mechanical

and chemical properties. Equally with colloidal GNP, carbon nanotubes have been used to construct or modify electrodes and have been applied to catalyze reduction or oxidation of various biomolecules electrochemically; they have shown superior performance compared with other carbon electrodes (Gooding 2005). Especially, they can penetrate inside redox enzymes and proteins and come close to the redox center of proteins, thus enhancing the direct electron transfer between the proteins and electrodes. Cytochrome c (Wang et al. 2002), catalase (Wang, Wang, and Zhou 2004), hemoglobin (Hb) (Cai and Chen 2004a; Yin et al. 2005), glucose oxidase(Cai and Chen 2004b), horseradish peroxidase(Xu et al. 2003), and myoglobin (Mb) (Zhao et al. 2003) in both single-walled carbon nanotubes (SWCNT) and multi-walled carbon nanotubes (MWCNT) films cast on electrode surfaces demonstrate well-defined and quasireversible cyclic voltammograms. MWCNT can be conveniently dispersed in Nafion solution on the basis of the special interactions between the sidewall of MWCNT and the hydrophobic domains of Nafion. Casting of the mixture on electrode surfaces produced uniform composite films, which have many electroanalytical applications.

Catalase (CAT) is a heme protein that belongs to the class of oxidoreductases with ferriprotoporphyrin-IX at the redox center, and it catalyzes the disproportionation of hydrogen peroxide into oxygen and water without formation of free radicals. In addition CAT is a redox enzyme that is present in all aerobic organisms (Chen et al. 2001). Electrochemical techniques have been a valuable tool for the study of CAT properties and have been applied to construction of an enzyme electrode. Direct electrochemistry of CAT provides a model for investigating mechanisms of redox transformations between enzyme molecules in biocatalysts and metabolic processes involving electron transportation in biological systems (Salimi Noorbakhsh, and Ghadermarzi 2007). Direct electrochemistry of CAT in polyacrilamide hydrogel film (Lu, Li, and Hu 2003), didodecyl-dimethylammonium bromide liquid crystal film (Chen et al. 2001), and gold electrode modified with SWCNT have been reported (Wang, Wang, and Zhou 2004). Based on the consideration that MWCNT and GNP might be the best candidates among various substrates for promoting the electron transfer reaction of CAT and for biomolcular attachment, we developed the possibility of performing voltammetric studies of CAT at MWCNT and GNP modified pyrolytic graphite (PG) electrodes.

In this work, a Nafion/CAT-GNP/MWCNT/PG electrode was prepared. GNP and MWCNT were attached onto the PG electrode surface through a Nafion monolayer and characterized by electrochemical impedance spectroscopy and cyclic voltammetry. We studied the direct electron transfer of CAT immobilized on MWCNT and GNP by a Nafion film, in which the GNP is used to retain the bioactivity of CAT

and facilitate the direct electron transfer between CAT and electrode. The biological and electrochemical activities of CAT were characterized with Ultraviolet-Visible (UV-Vis) spectroscopy and cyclic voltammetry. The immobilized CAT by GNP and MWCNT, acting as bridges of electron transfer, exhibits a fast direct electron transfer and retains its electrocatalytic behavior to hydrogen peroxide. The GNP and MWCNT attached to the PG electrodes were applied to the immobilization/adsorption of CAT with surface coverage of about 2.4×10^{-9} mol·cm^{-2} and consequently obtained the direct electrochemistry of CAT.

EXPERIMENTAL

Reagents

Catalase (EC 1.11.1.6) was purchased from Sigma and used without further purification. Chloroauric acid tetrahydrate ($HAuCl_4 \cdot 4H_2O$) and Nafion (5 wt.%) solution in a mixture of lower aliphatic alcohols were obtained from Aldrich. Phosphate buffer solutions, 0.1 M, with various pH values were prepared by mixing stock standard solutions of Na_2HPO_4 and KH_2PO_4 and adjusting the pH values with 0.1 M H_3PO_4 or NaOH solutions. All other chemicals were of analytical grade. All solutions were made with doubly distilled water.

Apparatus

Ultraviolet-Vis spectra were recorded on a UV-Vis spectrophotometer (HP8453). All electrochemical measurements were carried out with a CHI 660B electrochemistry work station (Shanghai CH Instruments, China) with a conventional three-electrode cell. The modified or unmodified PG electrode was used as the working electrode. The Pt wire and the saturated calomel electrode were used as the counter and reference electrodes, respectively. All solutions were purged with high purity nitrogen for at least 20 min prior to experiments and a nitrogen environment was then kept over the solution in the cell. Amperometric experiments were carried out in a stirred system by applying a potential step of 0.28 V to the working electrode. Aliquots of hydrogen peroxide standard solution were added successively to the solution. Current-time data were recorded after a steady state current had been achieved.

Electrochemical impedance measurements were performed in the presence of a 5 mM $K_3Fe(CN)_6$ solution pH 6.98 PBS containing 0.1 M KCl, using an alternating current voltage of 5 mV. Impedance

measurements were performed at a bias potential of 0.22 V, with the frequency range from 0.1 to 100,000 Hz.

Preparation Colloidal Gold and MWCNT

All glassware used in these preparations was thoroughly cleaned in aqua regia (3 parts $HCl + 1$ part HNO_3), and dried in air prior to use. Gold colloids were prepared according to literature (Frens 1973) with slight modification. The prepared GNP were stored in brown glass bottles at 4°C. MWCNT were pretreated with mixed HNO_3 and H_2SO_4 (1:3 volume ratio of 68%HNO_3 and 98% H_2SO_4) for 8 h at 70°C with ultrasonication to introduce carboxylic acid groups on the surface of the carbon nanotubes (Liu et al. 1998; Chattopadhyay, Galeska, and Papadimitrakopoulos 2001). After being washed thoroughly with water until the washing solution became neutral, the MWCNT were dispersed in 1% Nafion solution at a concentration of 1 mg· mL^{-1}.

Preparation of Nafion/CAT-GNP/MWCNT/PG Electrode

PG electrodes were polished first with 0.3- and 0.05-μm alumina slurry. After rinsing thoroughly with doubly distilled water, they were sonicated in absolute ethanol and doubly distilled water for about 5 mins. Catalase solution was obtained by dissolving 10.0 mg of CAT in 1 mL of 0.1 M pH 6.98 PBS, and the GNP were used as prepared. The Nafion/CAT-GNP/MWCNT/PG electrode was prepared by following procedure. First, a 6 μL mixture of MWCNT and 1% Nafion solution was dropped onto the surface of a cleaned PG electrode with a microsyringe and dried at room temperature. Then a 6 μL mixture of CAT and GNP (v/v = 1:1) was dropped onto the surface of MWCNT and Nafion and allowed to dry at ambient temperature without light. Finally, 4 μL of 1% Nafion solution was casted and used as a net to hold the CAT-GNP on the electrode surface stably. The solvent was allowed to evaporate before use. The resulting electrode was the Nafion/CAT-GNP/MWCNT/PG electrode. Similar procedures were employed to fabricate the Nafion/CAT/PG and Nafion/CAT/MWCNT/PG electrode. All prepared electrodes were stored at −20°C in 0.1 M pH 6.98 PBS when not in use.

RESULTS AND DISCUSSIONS

UV Studies of the Interaction Between CAT and GNP

Ultraviolet-Vis absorption spectra were performed to study the effects of GNP on the microstructures of CAT. The possible changes inside

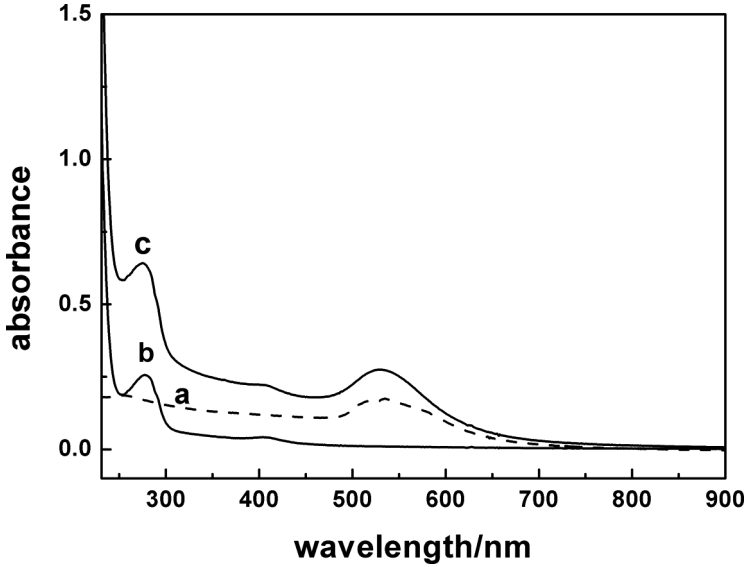

Figure 1. UV-Vis absorption spectra of (a) GNP solution, (b) CAT in 0.1 M pH 6.98 PBS, and (c) the mixed solution of CAT and GNP (v/v = 1:1).

the microenvironment of CAT could be inspected by the sensitivity of US-Vis. Figure 1 shows the US-Vis absorption spectra of (a) GNP, (b) CAT in 0.1 M pH 6.98 PBS, and (c) the mixture of CAT and GNP. As can be seen, the native state of CAT solution had an intense band at 280 nm. The peak could also be observed in the mixture of CAT and GNP and had nearly no difference in the site or shape, illuminating that the CAT keeps its natural structure in the mixture. In addition, the GNP solution exhibited a distinct surface plasmon absorption band at 519 nm. When mixed with CAT, there is nearly no change in the site. The result shows that GNP are uniformly dispersed in the mixture and the mixture does not show loss of biological activity of CAT.

Electrochemical Characteristics on the Modified Electrode Surface

Electrochemical impedance spectroscopy (EIS) is an effective method to probe the interfacial properties of modified electrodes, and understand chemical transformations (Wang and Peng 1986). In EIS, the semicircle part at higher frequencies corresponds to the electron transfer limited process. The semicircle diameter in the impedance spectrum corresponds to the electron transfer resistance, R_{et}. Fig. 2 shows the results of the EIS

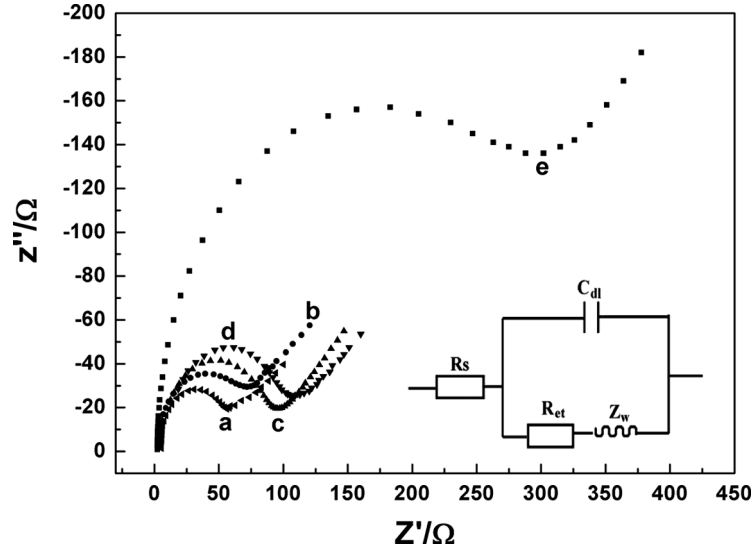

Figure 2. Electrochemical impedance spectroscopy of 5.0 mM $Fe(CN)_6^{3-}$ solution in pH 6.98 PBS containing 0.1 M KCl at a GNP/PG electrode (a), MWCNT/PG electrode (b), bare PG electrode (c), Nafion/CAT-GNP/MWCNT/PG electrode (d), Nafion/CAT/PG electrode (e). The frequency range is in 0.1–10^5 Hz at the formal potential of 0.22 V. The inset shows the equivalent circuit used to model impedance data.

at the MWCNT/PG electrode, GNP/PG electrode, bare PG electrode, Nafion/CAT-GNP/MWCNT/PG electrode, and Nafion/CAT/PG electrode in PBS (pH 6.98) containing 0.1 M KCl and 5.0 mM $Fe(CN)_6^{3-}$ solution. As shown in Fig. 2, two obvious semicircles were observed at the GNP/PG and MWCNT/PG modified electrodes (Fig. 2a, 2b) that were smaller than the bare PG electrode (Fig. 2c). The reason is that MWCNT and GNP could promote electron-transfer between CAT and electrode. After the bare PG electrode was coated with CAT and Nafion, the EIS of the resulting film shows a high interfacial R_{et} (Fig. 2e). The R_{et} decreased sharply after the MWCNT and GNP were modified on the PG electrode, which formed the Nafion/CAT-GNP/MWCNT/PG electrode (Fig. 2d).

The equivalent circuit for an electrode undergoing heterogeneous electron transfer is usually described on the basis of the model by Randles as shown in the inset of Fig. 2. The impedance data were fitted by the EvolCRT software. The circuit includes (1) the electrolyte resistance, R_s; (2) the double-layer capacitance, C_{dl}; (3) the electron transfer resistance, R_{et}; and (4) the Warburg element, Z_w. We used this equivalent

Table 1. Values of each element in the equivalent circuit for different modified electrode

Modified electrode	$R_s/\Omega\cdot cm^2$	$R_{ct}/\Omega\cdot cm^2$	$C_{dl}/\mu F\cdot cm^2$
Bare PG electrode	24.22	78.32	0.063
MWCNT/PG electrode	26.53	64.08	2.31
GNP/PG electrode	25.56	55.99	2.03
Nafion/CAT/PG electrode	26.63	312.26	2.75
Nafion/CAT-GNP/MWCNT/PG electrode	27.02	113.43	2.22

circuit to fit the impedance spectroscopy and determined C_{dl} and R_{et}. The values of the parameters in the circuit are shown in Table 1. The calculation results showed that R_{et} of the GNP/PG electrode, MWCNT/PG electrode, bare PG electrode, and Nafion/CAT/PG electrode were 55.99 Ω, 64.08 Ω, 78.32 Ω, and 312.26 Ω, respectively; after immobilization of CAT with MWCNT and GNP, R_{et} decreased to 113.43 Ω. Compared with R_{et} of the bare PG electrode, R_{et} of the Nafion/CAT-GNP/MWCNT/PG electrode clearly increased due to step-wise adsorption on the electrode surface, generating a tightly packed film and introducing a barrier to the interfacial electron transfer.

Direct Electron Transfer of CAT

The cyclic voltammograms of the bare PG electrode in PBS in the presence or absence of CAT in solution showed no response. However, a pair of well-defined and nearly reversible redox peaks for the direct electron transfer of CAT could be observed on Nafion/CAT-GNP/MWCNT/PG electrode in 0.1 M pH 6.98 PBS, as shown in Fig. 3d. The anodic peak potential (E_{pa}) and cathodic peak potential (E_{pc}) are located at -0.461 and -0.495 V, respectively, at a scan rate of 100 mV/s. The formal potential ($E^{o\prime}$), calculated from the average value of the anodic and cathodic peak potentials is -0.478 V, and the ratio of anodic to cathodic peak currents is about one. This indicates that CAT undergoes a quasi-reversible redox process (Fe (III)/Fe (II) redox couple) at the PG electrode modified with the modified film. This indicates that GNP and MWCNT have an effect on the kinetics of the electrode reaction for CAT and provide a favorable microenvironment for electron transfer of the CAT on the PG electrode.

In contrast, no peak was observable at the Nafion/GNP/PG electrode (Fig. 3a). When only CAT was entrapped in the Nafion film without the presence of GNP, the cyclic voltammogram showed a weak response of CAT (Fig. 3b). However, the response was much weaker than the

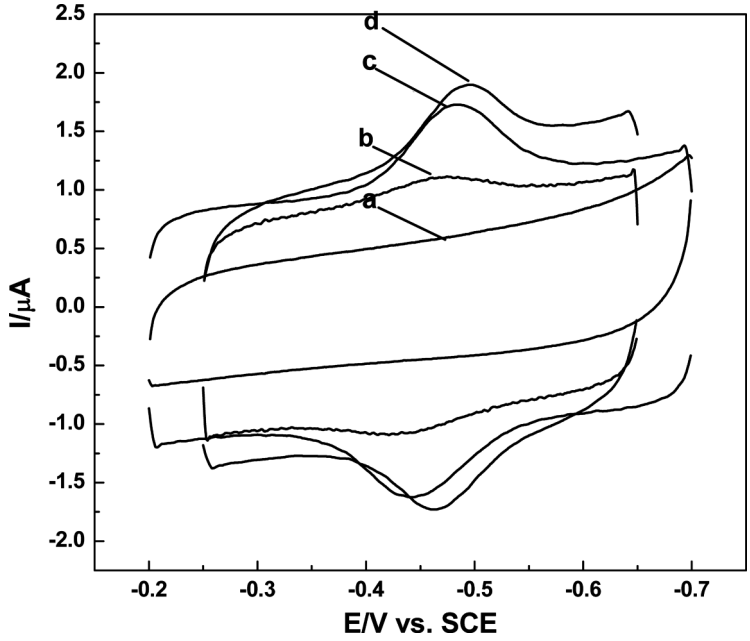

Figure 3. Cyclic voltammograms of (a) Nafion/GNP/PG electrode, (b) Nafion/CAT/PG electrode, (c) Nafion/CAT-GNP/PG electrode, and (d) Nafion/CAT-GNP/MWCNT/PG electrode in 0.1 M pH 6.98 PBS at scan rate of 100 mV/s.

Nafion/CAT-GNP/PG electrode (Fig. 3c). The Nafion/CAT-GNP/PG electrode also presented a pair of nearly reversible redox peaks with a little shift of peaks. Potential shift was due to the different film components, which may interact with CAT or affect the electric double layer of the electrode (Lu, Li, and Hu 2003; Shen and Hu 2004; Hung, Hu, and Zhou 2002; Li et al. 2005; Di et al. 2006). However, the Nafion/CAT-GNP/MWCNT/PG electrode kept a much stabler and stronger response than other modified eletrode. Thus, GNP and MWCNT played important roles in facilitating the electron (Fig. 3d). It is most likely that MWCNT and GNP have relatively larger specific surfaces to integrate enzyme and to aid in the orientation of the enzyme absorption as well. Simultaneously, they act as electron transfer tunnels, which may favor the electron transfer between the enzyme and electrode.

To obtain the kinetic parameters of CAT at the Nafion/CAT-GNP/MWCNT/PG electrode, the effect of scan rate was examined in 0.1 M PBS in the absence of oxygen. Figure 4 shows recorded cyclic voltammograms at different scan rates. Both the cathodic and anodic peak

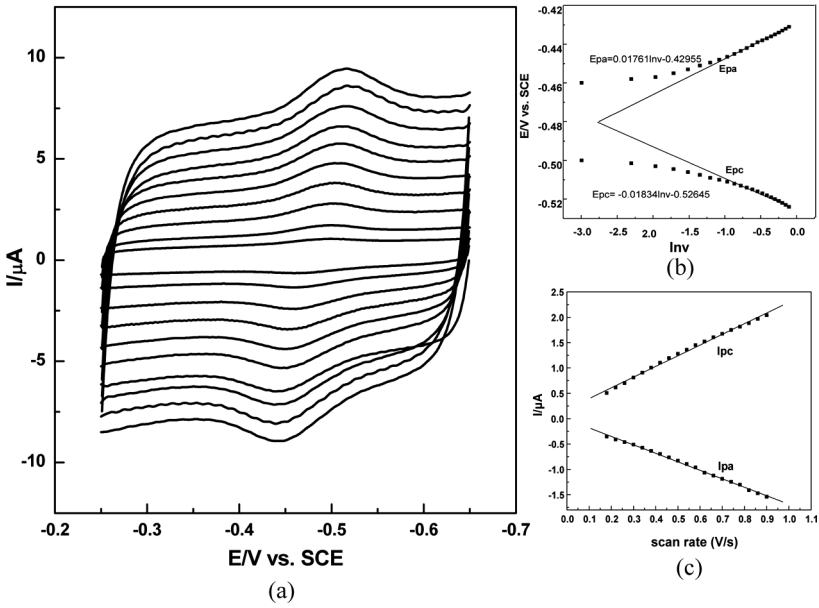

Figure 4. (a) Cyclic voltammograms of the Nafion/CAT-GNP/MWCNT/PG electrode in 0.1 M pH 6.98 PBS at various scan rates. The scan rates are 50, 100, 180, 260, 340, 420, 500, 580, 660, and 740 mV/s (from inner to outer). (b) Relationship between scan rate and the cathodic and anodic peak current. (c) plot of cathodic and anodic peak potential versus ln v. (SCE, saturated calomel electrode).

currents are linearly proportional to the scan rate in the range from 50 to 900 mV/s (linear regression equations: P_a: $i_{pa} = -1.67331 \times 10^{-6}v - 1.41405 \times 10^{-8}$, $r = 0.9986$; P_c: $i_{pc} = 2.10948 \times 10^{-6}v + 1.86334 \times 10^{-7}$, $r = 0.9976$), which indicates a surface-controlled electrode process.

Moreover, E_p remains a virtually small variety within the range of scan rates studied. It is clear that CAT adsorbed onto the surface undergoes a quasi-reversible electron transfer with interaction of nanoparticles. The peak to peak separation is about 40 mV at scan rates below 100 mV s^{-1}, suggesting facile charge transfer kinetics over this range of sweep rates. On the other hand, it is found that at a scan rate greater than 400 mV s^{-1}, ΔE_p increases by increasing the scan rate. The values of peak to peak potential separations are proportional to the natural logarithm of the scan rate for scan rate higher than 400 mV s^{-1} (Fig. 4b). The heterogenous electron transfer rate constant (k_s) of the CAT immobilized on the Nafion/CAT-GNP/MWCNT/PG electrode was estimated by the method of Laviron for a typical thin layer electrochemical system (Laviron 1979a, 1979b). The transfer coefficient (α) and heterogeneous

electron transfer rate constant of CAT (k_s) are about 0.49 and $1.387 \pm 0.1\ s^{-1}$, respectively. This value shows that the electron transfer of CAT on Nafion/CAT-GNP/MWCNT film is facile. It is assumed that the nanoparticles of the film increased the effective surface area, active points for adsorbing CAT, and also made the film more porous for facilitating electron transfer. In addition, the nanostructure of GNP and MWCNT may act as a molecular wire enhancing the direct electron transfer of CAT (Salimi et al. 2007). In fact, nanoparticles could play the role of an efficient electron conducting tunnel and have a very high ratio of surface to volume (Zhao et al. 1992). According to the slope of the I_p–v curve and the following equation, the surface concentration (Γ_c) of CAT on the surface of the modified PG electrode was estimated (Wang 1999).

$$I_P = n^2F^2vA\Gamma_c/4RT$$

where v is the sweep rate, A is the effective surface area of the modified electrode, and the other symbols have their usual meaning. From the slope of cathodic peak currents vs. scan rate the calculated surface concentration of CAT (Γ_c) is 2.4×10^{-9} mol·cm^{-2}, indicating a submonolayer of CAT on the Nafion/CAT-GNP/MWCNT/PG electrode.

Effect of pH on Peak Potential of CAT at Nafion/CAT-GNP/MWCNT/PG Film-Modified Electrode

The effect of pH values of the buffer solutions on the peak potentials of the Nafion/CAT-GNP/MWCNT/PG electrode was investigated by cyclic voltammetry. As shown in Fig. 5, both cathodic and anodic peak potentials of the Fe(III)/Fe(II) redox CAT shifted negatively with increasing pH values with a slope of 48 mV/pH in the range of pH 4–9. This slope is nearly the same as the theoretical value of 58 mV/pH at 20°C for reversible single-electron transfer coupled with single-proton transportation (Bond 1980).

Stability of Nafion/CAT-GNP/MWCNT/PG Electrode

Long-term stability is one of the most important properties required for a sensor, biosensor, or bioreactor. The direct electron transfer of CAT is stable. The stability of the Nafion/CAT-GNP/MWCNT/PG electrode was investigated by cyclic voltammetry. The amount of degradation after 150 cycles in PBS (pH 6.98) with a scan rate of 100 mV/s was less than 5% (Fig. 6). The peak current decreased with the increase of storage

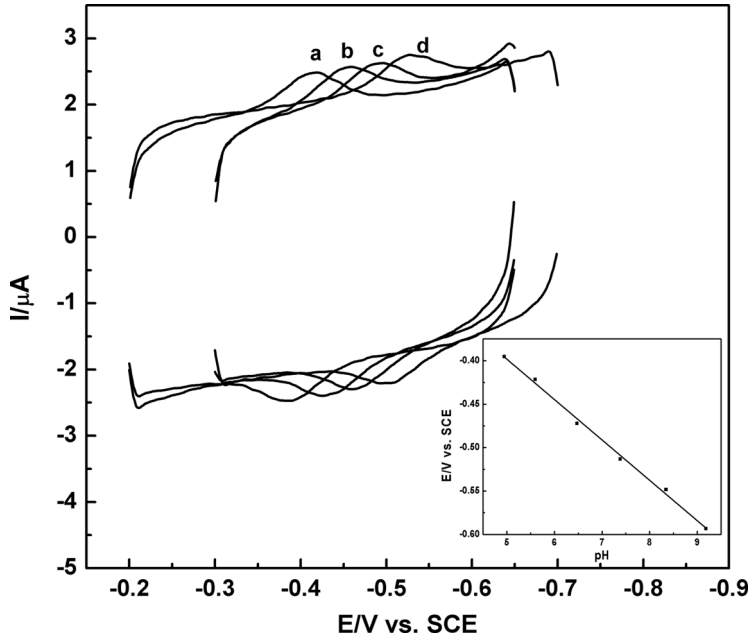

Figure 5. Cyclic voltammograms of modified Nafion/CAT-GNP/MWCNT/PG electrode in different pH solutions (from a to d: 5, 6.5, 7, and 9.2) (scan rate 100 mV/s). Inset: plot of formal potential versus pH values.

time. The stability of modified film was investigated by recording a cyclic voltammogram of modified electrode in PBS (pH 6.98). After it was stored at -20°C for 20 days, the CV peak potentials remained at the same positions, and the reduction peak currents decreased by only approximately 8%. Thus, the high stability of the modified electrode is related to the chemical stability of MWCNT film, the interaction between CAT and nanoparticles, and the strong adsorption of CAT on carbon nanotubes and GNP. Because of its long-term stability and excellent electron transfer rate constant, the Nafion/GNP-CAT/MWCNT/PG electrode can be used as a biosensor.

It was reported that enzymes and proteins containing heme groups are able to reduce hydrogen peroxide electrocatalytically. In order to verify whether the CAT immobilized on the modified PG electrode is denatured or not, the electrochemical experiments in the presence of hydrogen peroxide were carried out. Cyclic voltammetric experiments demonstrated that the immobilized CAT still retained its electrocatalytic activity. Figure 7 shows cyclic voltammograms of the modified electrode in the absence and presence of different concentrations of hydrogen

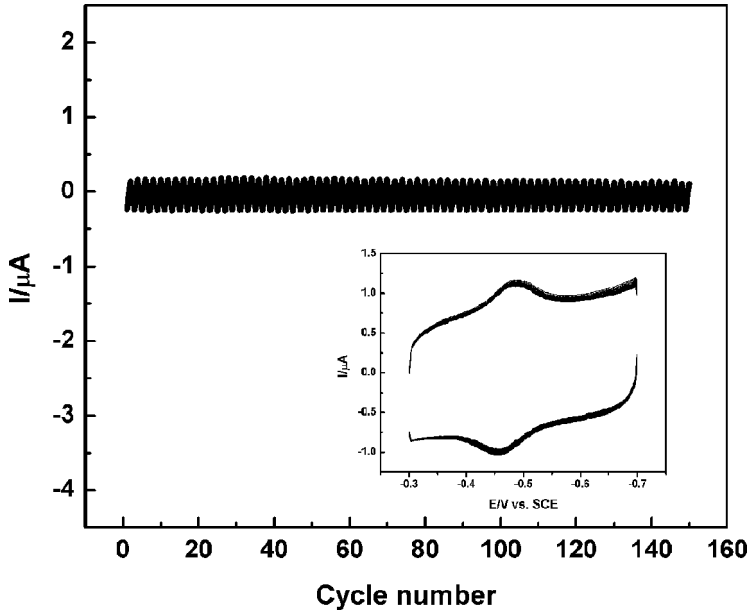

Figure 6. Stability of the Nafion/CAT-GNP/MWCNT/PG electrode on continuous scanning. The normalized peak current was calculated by comparing the response of the electrode with that of the first cycle. Inset: plot of 150 cycles cyclic voltammogram.

peroxide. For the bare PG electrode, there was no redox response of H_2O_2 in the potential range from -0.2 to -0.8 V (Fig. 7a). However, at the Nafion/CAT-GNP/MWCNT/PG electrode, the reduction current of enzyme film was greatly increased due to catalytic reduction of hydrogen peroxide, whereas the oxidation peak sharply disappeared. The decreased overvoltage and increased peak current of hydrogen peroxide reduction confirm that CAT has high catalytic ability for H_2O_2 reduction. For evaluating the activity of CAT immobilized on modified film, the i – t curve of the modified electrode in the presence of different concentrations of hydrogen peroxide was recorded in Fig. 8.

Figure 8 shows a typical current-time plot of the Nafion/CAT-GNP/MWCNT/PG electrode on successive step changes of hydrogen peroxide concentration. The constant potential of the modified electrode was set at -0.28 V after optimization, and the catalytic reduction current was monitored while aliquots of hydrogen peroxide were added. The Nafion/CAT-GNP/MWCNT/PG electrode reached 90% of the steady-state current within 200 s. The current response of the enzyme electrode increased along with the hydrogen peroxide concentration.

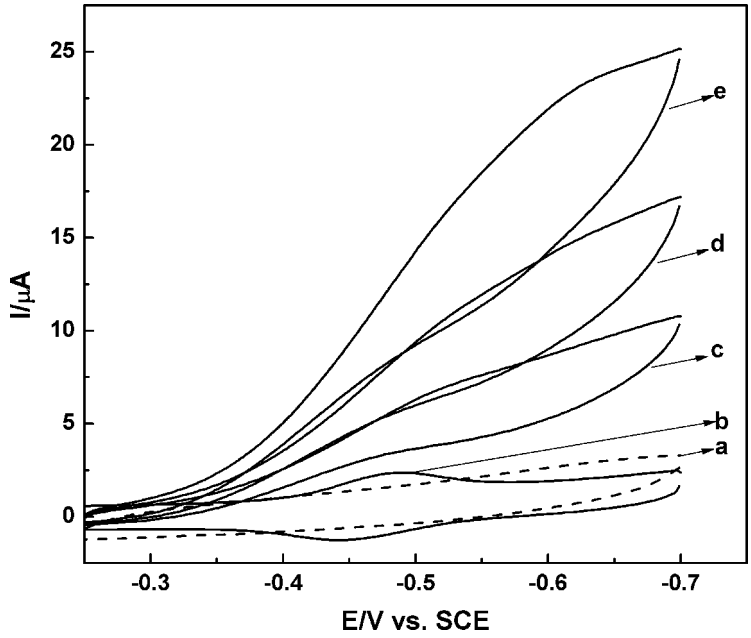

Figure 7. Cyclic voltammograms of the bare PG electrode in the presence of H_2O_2 (a) and the Nafion/CAT-GNP/MWCNT/PG electrode in 0.1 M pH 6.98 PBS (b) in the presence of different concentrations of H_2O_2 (c,d,e) at 100 mV/s.

Figure 8 (inset) shows a calibration plot of the steady-state current versus the hydrogen peroxide concentration. The linear response range of the Nafion/CAT-GNP/MWCNT/PG electrode to the concentration of hydrogen peroxide can be extended at least to 5 mM. When hydrogen peroxide concentration was high, a plateau current was observed, showing the characteristics of the Michaelis-Menten kinetics. The apparent Michaelis-Menten constant (K_M), as an important parameter to reveal enzyme-substrate reaction kinetics, can be obtained from the electrochemical version of the Lineweaver-Burk equation (Li, Tan, and Ge 1996).

$$1/I_{ss} = 1/I_{\max} + K_M/I_{\max} \cdot 1/C$$

where I_{ss} is the steady-state current after the addition of the substrate, C is the bulk concentration of the substrate, and I_{max} is the maximum current measured under saturated conditions. K_M can be obtained by the analysis of slope and intercept of the plot of the reciprocals of the steady-state current versus H_2O_2 concentration. The Michaelis-Menten

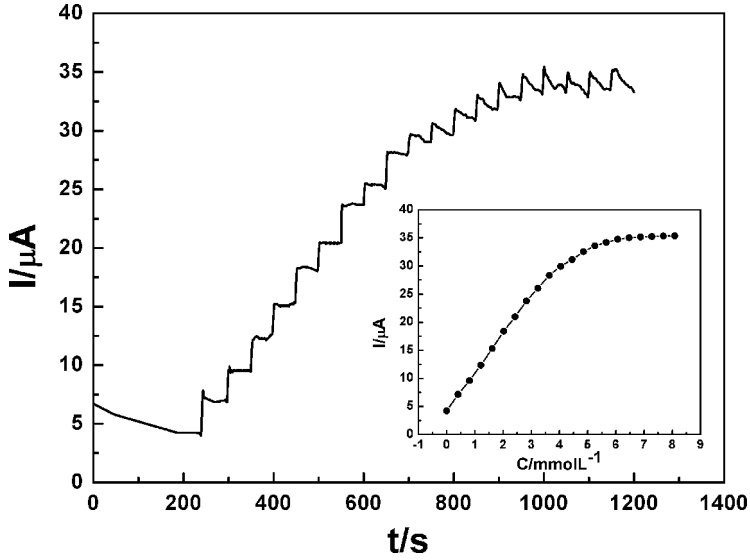

Figure 8. Current-time curves obtained at the Nafion/CAT-GNP/GC electrode for successive addition of different concentrations of hydrogen peroxide. Conditions: 0.1 M pH 6.82 PBS; applied potential, 0.29 V (vs. SCE). Inset: calibration curve of the sensor as a function of hydrogen peroxide.

constant of the system (K_M) in this work was found to be 1.71($\pm$0.05) mM, implying that the modified PG electrode exhibits a higher affinity for hydrogen peroxide. As is well known, the smaller K_M shows the higher catalytic ability.

CONCLUSIONS

In this paper, we realized the direct electron transfer of CAT by entrapping CAT-GNP/MWCNT in a Nafion film on a PG electrode. The properties of CAT entrapped in modified film were characterized by both spectroscopic and electrochemical techniques. Cyclic voltammetric results showed a pair of well-defined and nearly reversible redox peaks, which contribute to the direct electron transfer of CAT. The dependence of the formal potential on solution pH indicated that the direct electron transfer reaction of CAT was a single-proton coupled with single-electron redox reaction process. The experimental results further confirmed that the immobilized CAT retained its electrocatalytic activity for the oxidation of hydrogen peroxide. Therefore, the enzyme electrode can be used as an amperometric biosensor for the determination of hydrogen

peroxide. In addition, the biosensor also possesses high sensitivity and good chemical and mechanical stability.

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