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Direct Electrochemistry of Redox Proteins and Enzymes Promoted by Carbon Nanotubes

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Abstract: The redox protein and enzyme, such as hemoglobin (Hb), horseradish peroxidase (HRP) and glucose oxidase (GOx), was immobilized on the surface of the carbon nanotube modified glassy carbon (CNT/GC) electrode, respectively. The cyclic voltammetric results indicated that the redox protein and enzyme underwent effective and stable direct electron transfer reaction with a pair of nearly symmetrical redox peaks. The formal redox potential, $E^{0'}$, was almost independent on the scan rates, the average value of $E^{0'}$ for Hb, HRP and GOx was –0.343 ± 0.001, –0.319 ± 0.002 and –0.456 ± 0.0008 V (vs. SCE，pH 6.9), respectively. The dependence of $E^{0'}$ on the pH solution indicated that the direct electron transfer of Hb and HRP was a one-electron-transfer reaction process coupled with one-proton-transfer, while the GOx was a two-electron-transfer coupled with two-proton-transfer. The apparent heterogeneous electron transfer rate constant (k_s) was 1.25 ± 0.25, 2.07 ± 0.69 and 1.74 ± 0.42 s^{-1} for Hb, HRP and GOx, respectively. The method presented here can be easily extended to immobilize other redox enzymes or proteins and obtain their direct electrochemistry.

Keywords: carbon nanotube, direct electrochemistry, hemoglobin, horseradish peroxidase, glucose oxidase

1. Introduction

Electron transfer in biological systems is one of the leading areas of biochemical and biophysical sciences, and has been received more and more attention [1-9]. The direct electron transfer of enzymes (proteins) with electrodes can be applied to study enzymes-catalyzed reactions in biological systems

and this has developed into an electrochemical basis for the investigation of the structure of enzymes (proteins), mechanisms of redox transformations of enzyme (protein) molecules, and metabolic processes involving redox transformations. Enzyme-modified electrodes provide a basis for constructing biosensors, biomedical devices and enzymatic bioreactors. From these studies, one can find potential applications in biotechnology. For example, if an enzyme immobilized on an electrode surface is capable of the direct electron transfer and keeping its bioactivity, it can be used in biosensors and biofuel cells without the addition of mediators or promoters onto the electrode surface or into the solution. Unfortunately, it is difficult for an enzyme (a protein) to carry out the direct electrochemical reaction due to several factors. For example, enzymes (proteins) would be adsorbed on the electrode surface, resulting in the denaturation and loss of their electrochemical activities and bioactivities. In addition, usually, the larger three-dimensional structure of enzymes (proteins) and the resulting inaccessibility of the redox centers have made it generally difficult to obtain direct electron transfer between enzymes (proteins) and electrode surfaces, so that the promoters and mediators are needed to obtain their electrochemical responses. For the applications in biosensors, the enzymes (proteins) should be immobilized on the electrode surface to avoid many complications linked to the solution systems. Therefore, the suitable electrode materials and immobilization methods of enzymes (proteins) onto the electrode surface are important for obtaining their direct electrochemical reaction and keeping their bioactivities.

Since their initial discovery by Iijima [10] in 1991 and the subsequent report of the synthesis of large quantities by Ebbesen and coworkers [11] in 1992, carbon nanotubes (CNT) have been the major subject of numerous experimental and theoretical investigations [12]. Because of their novel structural and electronic properties, their high chemical stability, and their extremely high mechanical strength and modulus [12], CNT have found a wide range of potential applications from structural materials [13,14] to nanoelectronic components [15]. Specific applications of CNT include their use as a high sensitivity microbalance [16], gas detector [17,18], catalyst support [19,20], electron source in field emission mode for display [21], tiny tweezers for nanoscale manipulation [22] and probe tips for scanning probe microscopy [23]. Theoretical calculations have shown that, depending on its symmetry and diameter, CNT can be metallic or semiconducting [24,25]. The subtle electronic properties suggest CNT have the ability to promote electron transfer in electrochemical reactions when used as an electrode, representing a new application of CNT. The ability of CNT-modified electrodes to promote electron transfer reactions has been documented in connection to important biomolecules [26-34]. We have studied the direct electrochemical oxidation of dopamine and NAD(P)H [32,34] at a CNT-modified electrode. CNT can reduce the overpotential of electrochemical oxidation of NAD(P)H for 400 to 600 mV. Our goal is to explore new application of CNT as a new electrode material in facilitating the direct electron transfer between biomolecules and electrode. In this work, we report the direct electron transfer of hemoglobin (Hb), horseradish peroxidase (HRP) and glucose oxidase (GOx), which was immobilized onto the surface of CNT, respectively. The surface of CNT was covered with a layer of surfactant molecule, cetyltrimethylammonium bromide (CTAB, a cationic surfactant). The cyclic voltammetric results indicated that the direct electron transfer between the redox active center of immobilized Hb, HRP and GOx , respectively, and the surface of electrode occurred effectively.

2. Experimental

2.1 Chemicals

Hemoglobin (Hb, from Bovine Red Cells, Worthington Biochemical Corporation), horseradish peroxidase (HRP, EC 1.11.1.7, RZ > 3, 250 U/mg, Sigma), glucose oxidase (GOx, EC 1.1.3.4, from *Aspergillus niger*, 111 U/mg, Nanjing Sunshine Biotechnology Ltd., Nanjing, China), flavin adenine dinucleotide (FAD, disodium salt, 96%, Sigma) and hexaammineruthenium (III) chloride (99%, Strem Chemicals) were used as received. Nafion (10% in methanol with equivalent weight of about 1100) was obtained from Aldrich and was diluted to 5% with H_2O before use. Multi-wall carbon nanotube (CNT, <10-nm in diameter and 0.5 to 500-μm in length with the purity of >95%) was purchased from Shenzhen Nanotech Port Co. Ltd. (Shenzhen, China). All other chemicals were of analytical grade. All the solutions were prepared with doubly distilled water. 0.1 M phosphate buffer solutions (PBS, pH 6.9), which were made up from Na_2HPO_4 and NaH_2PO_4, were always employed as supporting electrolyte except that the pH-dependent experiments were carried out in PBS with various pH values.

2.2 Immobilization of enzymes and proteins on the surface of CNT

The GC electrode (4-mm in diameter) was polished sequentially with metallographic abrasive paper (No. 6), slurries of 0.3 and 0.05-μm alumina to a mirror finish. After rinsed with doubly distilled water, it was sonicated with absolute ethanol and then with doubly distilled water for about 1 min, respectively. CNT (1 mg) was dispersed in 1 mL CTAB (in water, 0.1% by weight, concentration greater than the critical micellar concentration, the critical micellar concentration of CTAB is 0.034% [35]) with aid of ultrasonication to give a 1-mg/mL stable black CNT suspension. CNT suspensions (2 μl) were mixed with 2 μL of Hb (5 mg/ml in PBS) thoroughly. Hb molecules were physically adsorbed onto the surface of CNT during mixing. Then 2 μL of the mixture was cast onto the surface of a GC electrode with a microsyringe and allowed to dry at ambient temperature. The orientation of CNT on the surface of GC electrode should be disorder, and CNT can physically interact with GC electrode via *van der Walls* forces. Finally, 1 μL of Nafion (5%) was cast and used as a binder to hold the Hb-CNT on the electrode surface stably. The solvent was allowed to evaporate before use. The final electrode is taken as Nafion-Hb-CNT/GC electrode. If not used immediately, the electrode was stored at 4 ˚C in a refrigerator. The same procedure was employed to fabricate the Nafion-HRP-CNT/GC and Nafion-GOx-CNT/GC electrode. The Nafion-CNT/GC electrode was also fabricated using the same procedures but without enzyme (or protein).

2.3 Apparatus

The scanning electron microscopic (SEM) images of CNT on electrode surface and the transmission electron micrograph (TEM) of CNT were obtained with a JEOL JSM-5610LV Scanning Electron Microscope (Japan) and a JEM-200CX Transmission Electron Microscope, respectively. FTIR spectra of CNT were recorded using a Nexus 670 FT-IR spectrophotometer (Nicolet Instrumental Co., USA) with the resolution of 4 cm^{-1}. The micro-Raman spectrum was recorded with a Spex 1403 Raman spectrometer at ambient temperature using 514 nm excitation and a spectral slit width of 2 cm^{-1}.

The electrochemical experiments were carried out with a CHI600 electrochemical workstation (CH Instruments, Shanghai Chenghua Co.) with a conventional three-electrode cell. A Nafion-CNT/GC, Nafion-Hb-CNT/GC, Nafion-HRP-CNT/GC or Nafion-GOx-CNT/GC electrode was used as the working electrode. The coiled Pt wire and the saturated calomel electrode (SCE) were used as the counter electrode and the reference electrode, respectively. Buffers were purged with high-purity nitrogen for at least 30 min prior to experiments and a nitrogen environment was then kept over solutions in the cell to protect the solution from oxygen. All experiments were performed at room temperature (22±2 ˚C).

The effective surface area of the Nafion-CNT/GC electrode was estimated using $Ru(NH_3)_6^{3+}$ as a probe. Cyclic voltammetric experiments of 1 mM $Ru(NH_3)_6Cl_3$ in 0.5 M KNO_3 solution at the Nafion-CNT/GC electrode were performed at various scan rates. A straight line of i_p versus $v^{1/2}$ can be obtained according to the following equation [36]:

$$i_p = 2.69\times10^5 n^{2/3} A D^{1/2} v^{1/2} c^* \quad (1)$$

Then, the effective surface area of the Nafion-CNT/GC electrode can be calculated using the slope of the line and by adopting the diffusion coefficient of 2.3×10^{-9} cm^2/s for $Ru(NH_3)_6^{3+}$ in Nafion film [37].

3. Results and discussion

3.1 Physical characterization

CNT are insoluble in most solvents [38,39] and especially in water. It has been reported, however, that the dispersity can be drastically improved by wrapping the CNT in polymeric chain such as poly(*p*-phenylenevinylene) [39], poly{(*m*-phenylenevinylene)-*co*-[2,5-dioctyloxy(*p*-phenylene)vinylene]} [40] or Nafion [41] etc., and by adsorption of surfactant molecules on the surface of CNT [42,43] without impairing the other physical properties [44,45]. When CNT were sonicated with CTAB solution, the CTAB molecule was adsorbed on the surface of CNT and created a distribution of positive charges that prevented the CNT aggregation and induced its suspension in water to form a stable black suspension. Such an improvement of dispersity of CNT in surfactant solution is clearly indicated by changes visible to the naked eye. The suspension of CNT in CTAB can be stable for a long time (at least one week). Fig. 1 is a TEM image of CNT. It can be concluded that the diameter of CNT is less than 10 nm and the wall of CNT is thin. And also, the surfaces of CNT, including inner and outer surface, are smooth.

Fig. 2 shows a SEM image of CNT on the surface of a GC electrode. It can be seen that the CNT are formed as bundles and some bundles are twisted together. The diameter of CNT bundles is in the range of 25 to 60 nm, the length of the bundle could not be measured since both ends of the bundle were not visible at the same time.

FTIR spectrum of CNT (not shown here) indicated that carboxylic (1715 cm^{-1}) and carboxylate (1574 cm^{-1}) groups are present on the surface of the CNT. The oxygen-contained group might be introduced during purification using concentrated nitric acid, and the carboxylate group might be attributed to the electroionization of the carboxylic group during washing with water after purification using HNO_3. The CNT were also characterized by Raman spectroscopy, which has been identified as a sensitive probe for the structure of carbon materials [46]. The Raman spectrum of CNT showed two

distinct peaks in the range of 1000 cm^{-1} to 2000 cm^{-1}. The Raman-allowed E_{2g} graphitic peak appears at 1585 cm^{-1} (G mode). The D band at 1340 cm^{-1} indicates the presence of amorphous carbon in the sample. The integrated relative intensities of the D mode versus the graphitic G mode showed that only a minor fraction of amorphous carbon is present in the sample, suggesting that the purity of CNT is high.

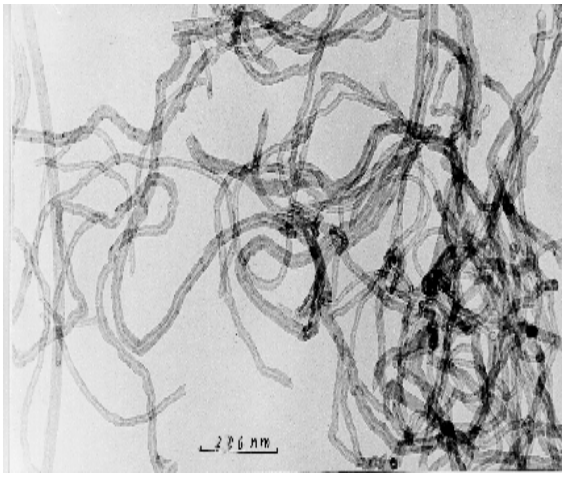

Figure1. TEM images of CNT.

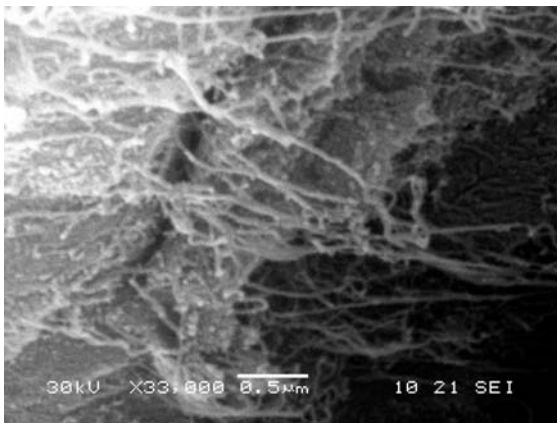

Figure 2. SEM images of CNT on the surface of GC electrode.

3.2 Direct electron transfer of Hb and HRP

Fig. 3 shows the cyclic voltammograms of a Nafion-CNT/GC electrode (curve a) and Nafion-Hb-CNT/GC electrode (curve b) in 0.1 M PBS (pH 6.9) at a scan rate of 60 mV/s. No any redox peaks was observed at the Nafion-CNT/GC electrode in the potential range of interest, but a pair of well-defined and nearly symmetrical redox peaks was obtained at the Nafion-Hb-CNT/GC electrode, suggesting that the redox peaks in curve b are ascribed to the electrochemical reaction of Hb immobilized on the surface of CNT. The anodic peak potential (E_{pa}) and cathodic peak potential (E_{pc}) are located at –0.312 and −0.372 V, respectively, at a scan rate of 60 mV/s. The separation of peak potentials, ΔE_p, is 60 mV, indicating that Hb immobilized on the surface of CNT displayed a quasi-reversible electrochemical reaction in spite of its large molecular structure. Its formal potential (defined as average of anodic and

cathodic peak potential), $E^{0'}$, is –0.342 V (at a 60 mV/s). The value of $E^{0'}$ is similar to that previously reported for Hb entrapping into polymer films, such as poly(vinyl sulfonate) (–0.337 V, pH 7.0) or Eastman AQ film (–0.341 V, pH 7.0) by Hu et al. [47,48], treating with dimethyl sulfoxide and adsorbed onto the surface of the pyrolytic graphite electrode (–0.360 V, pH 7.0) by Fan et al. [49]. It is also similar to those of other heme-containing proteins (enzymes) including myoglobin (–0.362 V, pH 7.0) [50], horseradish peroxidase (–0.363 V, pH 6.8) [51] and cytochrome $P450_{cam}$ (–0.354 V, pH 7.0) [52]. According to those reported previously, the electrochemical reaction in Fig. 3, curve b corresponds to the conversion of Hb-Fe(III) and Hb-Fe(II).

To verify either the direct electron transfer of Hb is facilitated by CNT or by the surfactant (CTAB) covered on the surface of CNT, following controlled experiments were performed. 2 μl Hb solution was mixed with 2 μl CTAB solution (without CNT) and 2 μl of the mixture was cast on the surface of the GC electrode and allowed to dry, then 1 μl of Nafion was applied. The resulting electrode did not show any observable electrochemical responses (curve d in Fig. 3). The result of curve c in Fig. 3, which shows the cyclic voltammogram of only Hb immobilized on a bare GC electrode surface using Nafion, indicated that the possibility that the electrochemical reaction of Hb at a bare GC electrode contributes to the observed redox waves in curve b of Fig. 3 should also be excluded.

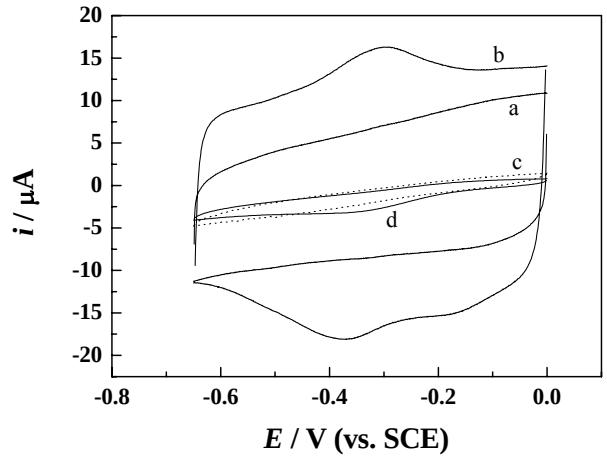

Figure 3. Cyclic voltammograms of the Nafion-CNT/GC (curve a) and the Nafion-Hb-CNT/GC (curve b) electrode in 0.1 M phosphate buffer solution (pH 6.9) at a scan rate of 60 mV/s. Curve c and d show the cyclic voltammograms of Hb (c) and Hb+CTAB (d), respectively, on the GC electrode. The scan rate is 60 mV/s.

The cyclic voltammetric results at various scan rates indicate that the values of E_{pa} and E_{pc} shift slightly to the positive and negative directions, respectively, and ΔE_p increases with increasing the scan rate. However, $E^{0'}$ is almost independent on the scan rates ($E^{0'}$ = –0.343 ± 0.001 V in the scan rate range of 20 to 120 mV/s). The anodic and cathodic peak currents are linearly proportional to scan rate up to more than 120 mV/s, suggesting that the reaction is not a diffusion-controlled process but a surface-controlled one, as expected for immobilized systems [36]. From the dependence of ΔE_p on the various scan rates, the apparent heterogeneous electron transfer rate constant, k_s, can be calculated to be 1.25 ± 0.25 s^{-1}, using the method developed by Laviron [53] for a surface-controlled

electrochemical system. The average Γ value of $(5.74 \pm 0.57)\times10^{-12}$ mol/cm^2 was obtained at a scan rate of 20 to 120 mV/s. According to the procedure of fabrication of Nafion-Hb-CNT/GC electrode (see Experimental), the total amount of Hb on the surface of electrode is 1.05×10^{-11} mol/cm^2, thus, the electroactive amount obtained here account for about 54.7%, suggesting that only a part of Hb presented on the electrode surface undergoes the direct electron transfer reaction. The fractions of electroactive Hb obtained here is much larger than that of immobilizing Hb in surfactant or polymer films (about 5 to 12%) [48,54,55], indicating that CNT is much effective in promoting the direct electron transfer of Hb.

The cyclic voltammetric characteristics of the Nafion-HRP-CNT/GC electrode are similar to that of the Nafion-Hb-CNT/GC electrode (Fig. 4). The anodic (E_{pa}) and cathodic peak potential (E_{pc}) are –0.300 and –0.339 V, respectively, at a scan rate of 60 mV/s. The separation of peak potentials, ΔE_p, is 39 mV and the formal potential is –0.320 V. The value of $E^{0'}$ is similar to that previously reported for HRP entrapped in the tributylmethyl phosphonium chloride bound to an anionic exchange resin (–0.38 V, pH 7.0) by Ferri et al. [56,57], incorporated into Eastman AQ film (–0.33 V, pH 7.0) by Huang and Hu [58] and immobilized onto the surface of active carbon powder (Vulcan XC-72) (–0.363 V, pH 6.8) [51]. The average value of $E^{0'}$ is (-0.319 ± 0.002) V in the scan range of 20 to 100 mV/s. The value of k_s is calculated to be (2.07 ± 0.56) s^{-1}. The average Γ value of $(1.90 \pm 0.44)\times10^{-12}$ mol/cm^2 was obtained at a scan rate of 20 to 100 mV/s and corresponding to 24.0% of total amount of HRP on the surface of electrode. These results indicate that CNT can also facilitate the direct electron transfer of HRP effectively.

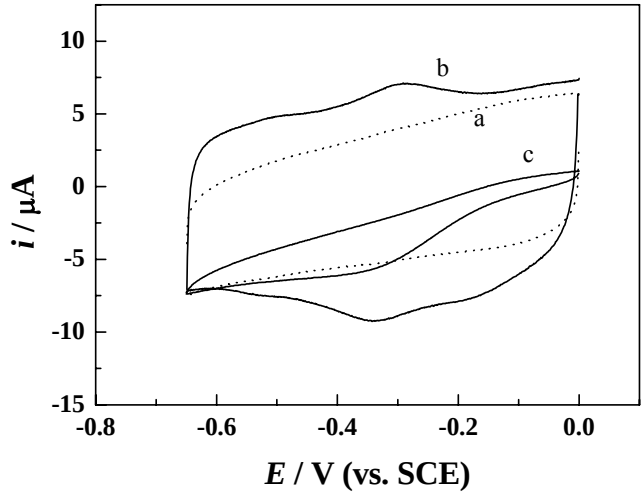

Fig.4 Cyclic voltammograms of the Nafion-CNT/GC (curve a), Nafion-HRP-CNT/GC (curve b) and Nafion-HRP-CTAB/GC (curve c) electrode in 0.1 mol/L PBS (pH 6.9) at a scan rate of 60 mV/s.

Most protein solution contains macromolecular impurities. Adsorption of protein and other macromolecules onto bare electrodes, often with denaturation, can create an insulating layer and inhibit the passage of electrons [59]. Thus, direct electron transfer of proteins was usually obtained only in highly purified solution. So, we propose that CNT, whose surface was covered with a layer of surfactant, modifying on the surface of electrode may inhibit adsorption of macromolecules, which

would otherwise block the electron transfer between protein (enzyme) and electrode, and thus help to facilitate the direct electron transfer. The reason that the CNT can facilitate the direct electron transfer of protein (enzyme) may partially be due to some oxygen-contained groups [27] present on the CNT surface, its small dimension, its electronic structure and high electrical conductivity, even though the exact reason is not fully clarified at present time. It was reported that CNT can promote the direct electron transfer of Cytochrome c [28], and catalyze the oxidation of catecholamines [60] and NAD(P)H [32,34] are also due to the presence of the oxygen-contained groups on its surface.

3.3 Direct electron transfer of GOx

Fig. 5, curve a is the cyclic voltammogram of a Nafion-CNT/GC electrode in 0.1 M PBS (pH 6.9) at a scan rate of 40 mV/s and curve b is the cyclic voltammogram of a Nafion-GOx-CNT/GC electrode in the same buffer solution and the same scan rate as that for curve a. It can be concluded that a pair of well-defined and nearly symmetric redox peaks was observed at a Nafion-GOx-CNT/GC electrode. After incubating the Nafion-GOx-CNT/GC electrode in 3 M guanidine hydrochloride solution overnight, the redox peak disappeared. Treatment of the Nafion-GOx-CNT/GC electrode with concentrated salt solution can easily strip the FAD active center from GOx molecule and/or remove the adsorbed GOx from electrode surface [61-63], though it is relatively ineffective in removing adsorbed free FAD from the electrode surface [62,63]. These results suggest that the redox peaks in curve b of Fig. 5 can be ascribed to the redox reaction of the prosthetic group (FAD) bound to the GOx [61,63] and not to free FAD, which may have dissociated away from GOx due to conformational changes during immobilization. Controlled experimental results indicated that the voltammetric responses of the Nafion-FAD-CNT/GC electrode remained a 68% of the initial one after the electrode was incubated 2 days in 3 M guanidine hydrochloride solution and still remained 24% even incubated 12 days.

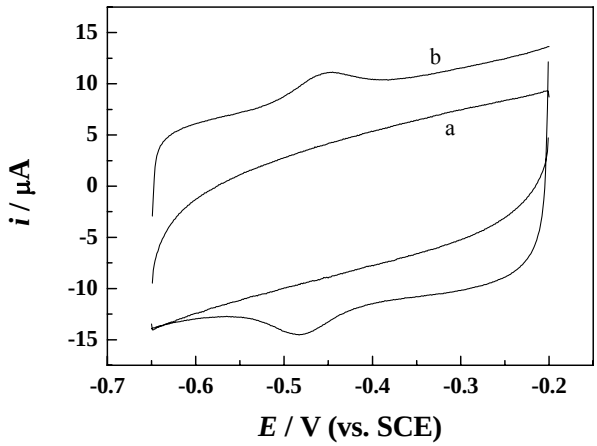

Figure 5. Cyclic voltammograms of the Nafion-CNT/GC (a) and Nafion-GOx-CNT/GC (b) electrode in 0.1 M PBS (pH 6.9) at a scan rate of 40 mV/s.

The anodic and cathodic peak potential of curve b in Fig. 5 is –0.445 V and –0.483 V (at 40 mV/s), respectively. The separation of peak potentials is small (ΔE_p = 38 mV), although not zero. The value of

$E^{0'}$ is –0.464 V at a scan rate of 40 mV/s. The anodic and cathodic peak currents are linearly proportional to scan rate up to more than 100 mV/s, suggesting the reaction is not a diffusion-controlled process but a surface-controlled one, as expected for immobilized systems [36].

To verify that the direct electron transfer of GOx is promoted by CNT itself and not by the surfactant covered on the surface of CNT, following experiments were performed. 2 μl of GOx solution was mixed with 2 μl of CTAB solution (without CNT) thoroughly. 2 μl of the mixture was cast on the surface of GC electrode and allowed to dry at ambient temperature. Then, 1 μl of Nafion (5%) was applied and the solvent was allowed to evaporate. The resulted electrode did not show any observable electrochemical responses (not shown here), suggesting the direct electron transfer of GOx is promoted by CNT, not by surfactant. The result also indicated that only GOx adsorbed on the surface of CNT can undergo the direct electron transfer, and the physically entrapped GOx (not adsorbed on the surface of CNT) cannot undergo a direct electron transfer.

The anodic and cathodic peak potentials of direct electron transfer of GOx are scan rate-dependent (Fig. 6). The anodic and cathodic peak potential shifts to positive and negative direction, respectively, and consequently, the ΔE_p increases with increasing of the scan rate. However, the value of $E^{0'}$ is independent on the scan rates ($E^{0'}$ = -0.466 ± 0.001 V in the scan rate range of 10 to 140 mV/s). From the dependence of ΔE_p on the scan rates, the apparent heterogeneous electron transfer rate constant, k_s, can be calculated to be 1.53 ± 0.45 s^{-1}. This value of k_s is much larger than that obtained by Jiang et al. (0.026 s^{-1}) [61] using the same method at a monolayer of 3,3'-dithiobissulfosuccinimidyl propionate (DTSSP) modified gold electrode, suggesting CNT is more effective in facilitating the direct electron transfer of GOx than DTSSP.

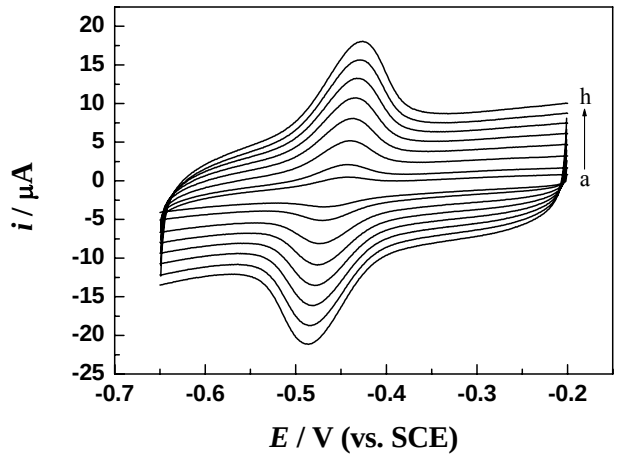

Figure 6. Cyclic voltammograms of the Nafion-GOx-CNT/GC electrode in 0.1 M PBS (pH 6.9) at various scan rates. The scan rate (from a to h) is 10, 20, 40, 60, 80, 100, 120 and 140 mV/s, respectively.

3.4 Effect of solution pH

It is known that solution pH modulates the accessibility of water to the heme pocket of Hb and HRP and the protonation of the heme iron-bound proximal histidine and/or the distal histidine in the heme

pocket and accordingly influences the redox potential of Hb [64]. Fig. 7 shows that the dependence of E_{pa}, E_{pc} and $E^{0'}$ of the Nafion-Hb-CNT/GC electrode on the solution pH. The increase of the value of pH of the solution leads to a negative shift of E_{pa}, E_{pc} and $E^{0'}$. All the E_{pa}, E_{pc} and $E^{0'}$ have a linear relationship with solution pH with a slope of –54.7, –56.0 and –55.4 mV/pH unit, respectively, in the range of 5.5 to 9.3. The dependence of E_{pa}, E_{pc} and $E^{0'}$ of the Nafion-HRP-CNT/GC electrode on the solution pH is similar to that of Nafion-Hb-CNT/GC electrode. The slope for the linear plot of E_{pa}, E_{pc} and $E^{0'}$ of HRP on pH is –63.3, –57.1 and –60.2 mV/pH. Those values are close to the –58.5 mV/pH unit expected for a reversible, one-electron coupled one-proton reaction process at 22 ˚C.

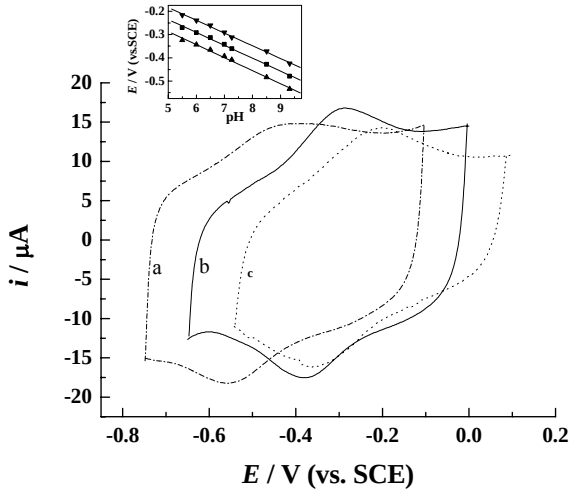

Figure 7. Cyclic voltammograms of the Nafion-Hb-CNT/GC electrode in 0.1 M phosphate buffer solution at pH of (a) 9.3, (b) 7.0 and (c) 5.5. The scan rate is 100 mV/s. The inset shows the dependence of E_{pa} (▼), E_{pc} (▲) and $E^{0'}$ (■) on the solution pH.

The electrochemical response of GOx immobilized onto the heterogeneous surface is due to the redox reaction of FAD [65], which is bound to the enzyme molecule. FAD is known to undergo a redox reaction of two-electron coupled with two-proton. Thus, the anodic and cathodic peak potentials of GOx immobilized on the surface of CNT should be pH-dependent. Fig. 8 presents such a plot. An increasing of the solution pH leads to a negative shift in potential for both anodic and cathodic peaks. The slope for linear plot of $E^{0'}$ vs. pH is –53 mV/pH, which is close to the theoretical one (–58.6 mV/pH) at 22 ºC for a reversible, two-proton coupled with two-electron redox reaction process.

4. Conclusions

The promotion effects of CNT (dispersed in 0.1% CTAB) on the direct electron transfer of hemoglobin (Hb), horseradish peroxidase (HRP) and glucose oxidase (GOx), which was immobilized onto the surface of CNT, respectively, have been reported. Cyclic voltammetric results showed a pair of well-defined redox peaks, which corresponded to the direct electron transfer of Hb, HRP and GOx, respectively. The dependence of $E^{0'}$ on the pH of the buffer solution indicated that the direct electron transfer of Hb and HRP was a one-electron-transfer reaction process coupled with one-proton-transfer,

while the GOx was a two-electron-transfer reaction process coupled with two-proton-transfer. The method presented here can be easily extended to immobilize and obtain the direct electrochemistry of other enzymes or proteins.

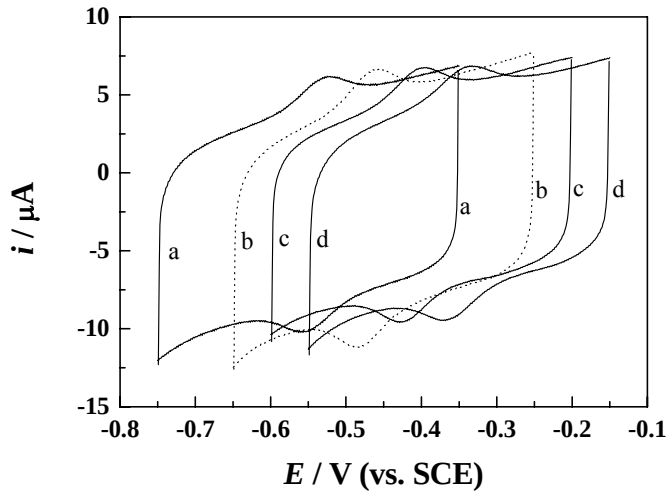

Figure 8. The dependence of cyclic voltammograms of the Nafion-GOx-CNT/GC electrode in 0.1 M PBS on solution pH. The solution pH for a to d is 8.5, 7.0, 6.0 and 4.5, respectively. The scan rate is 20 mV/s.

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