ELECTROCATALYSIS OF HEMOGLOBIN IN IONIC LIQUID BMIMPF$_6$ AND CuS NANOSPHERE COMPOSITE FILMS

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ABSTRACT. Ionic liquid 1-butyl-3-methylimidazolium hexafluorophosphate (BMIMPF$_6$) was mixed homogeneously with nanometer-sized semiconductor CuS sphere to form a new nanocomposite material, which was further used for the immobilization of hemoglobin (Hb) on the surface of carbon paste electrode (CPE). Direct electrochemistry of Hb in BMIMPF$_6$-CuS composite film was carefully investigated with a pair of quasi-reversible redox peaks appeared and the formal potential (E$^{0'}$) was got as -135 mV (vs. SCE) in pH 7.0 phosphate buffer solution, which was due to the enhanced direct electron transfer rate of Hb in the biocompatible matrix. The BMIMPF$_6$-CuS-Hb/CPE showed excellent electrocatalytic activity to the reduction of hydrogen peroxide with the kinetic parameters for the electrocatalytic reaction evaluated. The results indicated that the BMIMPF$_6$-CuS nanocomposite could be used for the preparation of electrochemical biosensor.

KEY WORDS: Hemoglobin, Direct electrochemistry, Copper sulfide nanosphere, 1-Butyl-3-methylimidazolium hexafluorophosphate, Hydrogen peroxide

INTRODUCTION

Direct electrochemistry of redox proteins on different kinds of electrodes have been widely studied for the potential applications in biosensor and bioreactor [1]. Due to the difficult of direct electron transfer (DET) between proteins and the bare electrodes, film modified electrodes have been used as an effective tool for the investigation. Various substances such as insoluble surfactants [2], hydrogel [3], biopolymer [4], composite film [5] or nanoparticles [6] had been explored as the immobilization matrix. Proteins can retain their native structure in the film and the DET rate between protein and electrode can be greatly enhanced with enhancer and/or modifier present on the electrode surface.

Recently room temperature ionic liquids (RTILs) have been widely used in the protein electrochemistry. RTILs are ionic compounds composed of organic cations and various anions. As a non-aqueous polar solvent, RTILs have many unique properties such as high chemical and thermal stability, relatively high ionic conductivity, negligible vapor pressure and wide electrochemical windows [7-9], so they have been widely used in different branches of chemistry [10, 11]. Enzyme can retain its bioactivity in RTILs and an enzyme coated by RTILs shows high selectivity, fast reaction rate and great enzyme stability, which can be further used in biocatalysis. In the field of electroanalytical chemistry, RTILs can be used as not only supporting electrolyte but also the modified materials. Maleki *et al.* applied N-octylpyridinum hexafluorophosphate as a binder for the construction of a high-performance carbon composite electrode and carefully investigated its electrocatalytic ability to different electroactive compounds [12]. The RTIL modified electrodes had been used for the protein electrochemistry or electroactive substances detection [13-15]. RTILs had also been mixed with biopolymers such as chitosan [16], sodium alginate [17] and sol-gel [18] to form a composite material that could be applied to the redox protein electrochemistry.

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With the fast development of nanotechnology, spherical nano-structural materials had aroused great interests due to their unique structural, optical and surface properties [19]. Nanospheres had many potential applications in the filed of chemistry, biotechnology and materials science [20] and could be acted as artificial cells, shape-selective adsorbents, light-weight fillers, catalyst or waste removal [21, 22]. Various kinds of inorganic or polymer materials with spherical structure have been synthesized by using layer-by-layer (LBL), self-assembly, microemulsion and solid state metathesis [23, 24]. Among them, copper sulfide (CuS) nanosphere had also been fabricated and used as the thermoelectric cooling material, optical recording material, solar cell or supersonic materials [25, 26].

In this paper nanometer-sized semiconductor CuS spherical material was mixed with 1-butyl-3-methylimidazolium hexafluorophosphate (BMIMPF$_6$) to form a composite film, which was further modified on the carbon paste electrode (CPE). Then hemoglobin (Hb) was immobilized in the film and the direct electrochemistry of Hb in the matrix was studied. The results showed that direct electron transfer of Hb was realized in the nanocomposite film, which could be attributed to the synergetic contribution of the high ionic conductivity of BMIMPF$_6$ with the specific properties of CuS nanosphere. The BMIMPF$_6$-CuS-Hb/CPE showed good electrocatalytic ability to the reduction of hydrogen peroxide (H$_2$O$_2$) with the parameters calculated.

EXPERIMENTAL

Chemicals

Bovine hemoglobin (Hb, MW 64500, Tianjin Chuanye Biochemical Limited Company, China), ionic liquid 1-butyl-3-methylimidazolium hexafluorophosphate (BMIMPF$_6$, Hangzhou Kemer Chemical Limited Company, China) and graphite powder (average particle size of 30 μm, Shanghai Colloid Chemical Plant, China) were used as received. CuS nanosphere (300 nm) was prepared according to a soft-template method [27]. 0.1 M phosphate buffer solutions (PBS) with various pH values were used as the supporting electrolyte. All the other reagents were of analytical reagent grade and doubly distilled water was used throughout.

Apparatus

Cyclic voltammetry was carried out on a CHI 840B electrochemical workstation and electrochemical impedance spectroscopy (EIS) was performed on a CHI 750B electrochemical workstation (Shanghai CH Instrument, China). A traditional three-electrode system was employed with a BMIMPF$_6$-CuS-Hb/CPE as working electrode (Φ = 4.2 mm), a saturated calomel electrode (SCE) as reference electrode and a platinum wire as auxiliary electrode. Scanning electron microscopy (SEM) and transmission electron microscopy (TEM) of CuS nanosphere were obtained by a JSM-6700F scanning electron microscope (Japan Electron Company, Japan) and JEM-2000 EX transmission electron microscope (JEOL Ltd., Japan), respectively. All the experiments were performed at room temperature. The tested solution was deoxygenated by highly purified N$_2$ for 30 min before the experiments and the electrochemical cell was kept at N$_2$ atmosphere during the experiments.

Procedure

The traditional carbon paste electrode (CPE) was fabricated by grinding a mixture of graphite powder with paraffin at a ratio of 70/30 (w/w) in an agate mortar. The homogeneous paste was packed into a cavity of glass tube with the diameter of 4.2 mm. A copper wire was inserted through the opposite end of the tube to establish an electrical contact and the surface of CPE

was carefully polished on weighing paper just before use. The mixture of 5.0 mg Hb, 50 μL BMIMPF$_6$ and 1.5 mg CuS nanosphere were ground in an agate mortar for 30 min to form a gel-like nanocomposite material. Then 2 μL of the mixture was cast onto the surface of the CPE to form a BMIMPF$_6$-CuS-Hb modified CPE. For comparison, other modified electrodes were prepared by the similar procedure.

RESULTS AND DISCUSSION

Images of CuS nanosphere

Figure 1 A and B show the TEM and SEM images of the CuS nanosphere, respectively. The results indicated that CuS nanosphere had a good dispersion with the average diameter about 300 nm.
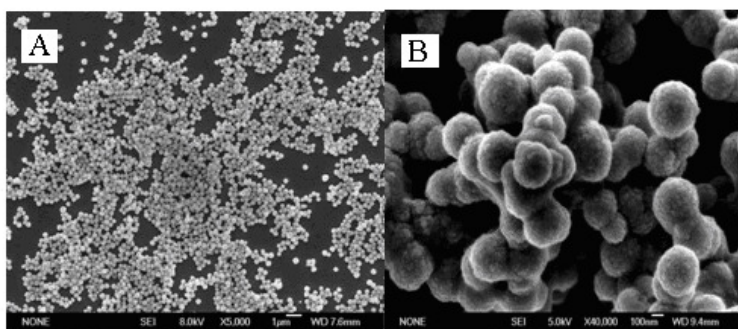

Figure 1. The typical TEM (A) and SEM (B) images of the CuS nanosphere.

Electrochemical impedance spectroscopy

Electrochemical impedance spectroscopic (EIS) experiment can provide the impedance changes of modified electrode interface. EIS measurement was performed in a mixture solution of 0.1 M KCl and 5.0×10^{-3} M [Fe(CN)$_6$]$^{3-/4-}$ by applying an AC voltage with 5 mV amplitude in the frequency range swept from 10^4 to 0.5 Hz under open circuit potential condition. Figure 2 showed the EIS results of different modified electrodes and the semicircle diameter of Nyquist plots reflected the electron transfer resistance (Ret) on the electrode surface. On traditional CPE the Ret value was got as 1305 Ω (curve a), which was attributed to the presence of nonconductive paraffin. While on BMIMPF$_6$ modified CPE, the Ret value was decreased to 932 Ω (curve b), which indicated the presence of good ionic conductivity of IL on the CPE surface. On BMIMPF$_6$-CuS/CPE, the Ret value was further decreased to 300 Ω (curve c), which was attributed to the presence of CuS nanosphere in the IL film. CuS nanoparticle is a semiconductor material with unique electronic properties, which can further enhance the conductivity of the composite film on the electrode surface. While on BMIMPF$_6$-CuS-Hb/CPE, the Ret value was increased to 1109 Ω (curve d), indicating the presence of Hb in the composite film on the electrode surface increased the resistance to the redox probe of [Fe(CN)$_6$]$^{3-/4-}$. The results indicated that different modification procedures on the electrode resulted in different electron transfer resistances and Hb had been successful immobilized on the electrode surface.
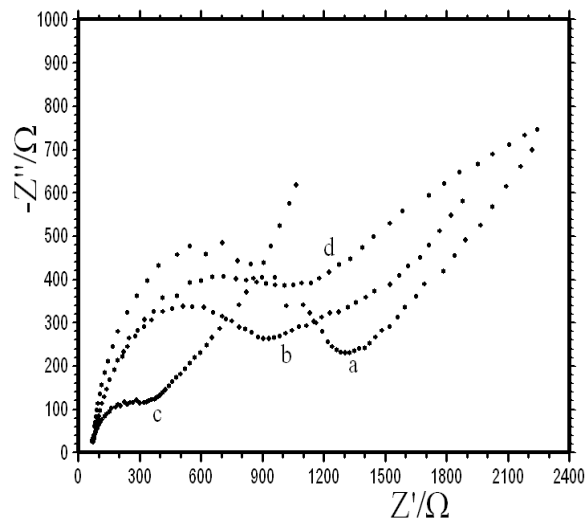
Figure 2. Nyquist diagram for the EIS responses at different modified electrodes (a) CPE, (b) BMIMPF$_6$/CPE, (c) BMIMPF$_6$-CuS/CPE and (d) BMIMPF$_6$-CuS-Hb/CPE in a 5.0 × 10^{-3} M [Fe(CN)$_6$]$^{3-/4-}$ + 0.1 M KCl solution with the frequencies range from 10^4 to 0.5 Hz.

Direct electrochemistry of Hb

Direct electrochemistry of Hb in the composite film was carefully studied in pH 7.0 PBS within the potential range from 0.3 to -0.8 V (vs. SCE). Figure 3 shows the typical cyclic voltammograms of different electrodes. On bare CPE (curve a), BMIMPF$_6$/CPE (curve b) and BMIMPF$_6$-CuS/CPE (curve c) no obvious redox peaks appeared, which indicated no electroactive substances existed on the electrode surface. While on BMIMPF$_6$-CuS-Hb/CPE a pair of well-defined quasi-reversible cyclic voltammetric peaks appeared (curve d) with the redox peak potentials located as E$_{pa}$ = -0.05 V and E$_{pc}$ = -0.22 V (vs. SCE), which could be ascribed to the presence of Hb in the composite film and the direct electron transfer of Hb in the BMIMPF$_6$-CuS composite with the underlying electrode was realized. CuS nanosphere has the advantages of large surface area, tunable porosity and high stability, which can be used as the support to assembly the Hb molecules and form a biocompatible porous hybrid complex. Meanwhile ILs possess the characteristics including higher ionic conductivity, wider electrochemical windows, better biocompatibility and inherent catalytic ability. So the composite film showed an improved capability to enhance the electron transfer rate of redox proteins. The formal potential (E$^{0'}$) of Hb is calculated by the equation of E$^{0'}$ = (E$_{pc}$ + E$_{pa}$)/2 and the result was got as -135 mV, which was similar to the references [28, 29]. The peak-to-peak separation (ΔE$_p$) was calculated as 170 mV and the value of I$_{pa}$/I$_{pc}$ was 0.91 at the scan rate of 100 mV s^{-1}. All the results indicated a quasi-reversible electrode process was taken place under the selected conditions.
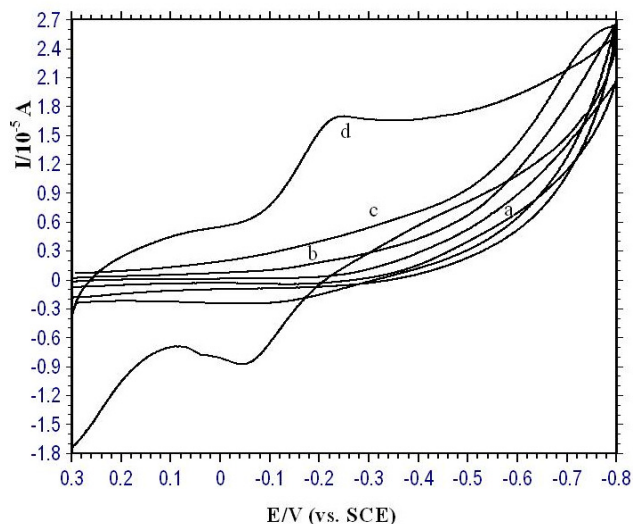
Figure 3. Cyclic voltammograms of (a) CPE, (b) BMIMPF$_6$/CPE, (c) BMIMPF$_6$-CuS/CPE and (d) BMIMPF$_6$-CuS-Hb/CPE in pH 7.0 PBS at the scan rate of 100 mV s^{-1}.

The influence of scan rate on the redox peak response was investigated with the results shown in Figure 4. It can be seen that cyclic voltammetric peak currents were increased gradually with the increase of scan rate. A linear relationship was got between the redox peak current and the scan rate from 100 to 500 mV s^{-1}, which was a typical diffusionless and surface-confined voltammetric behavior.
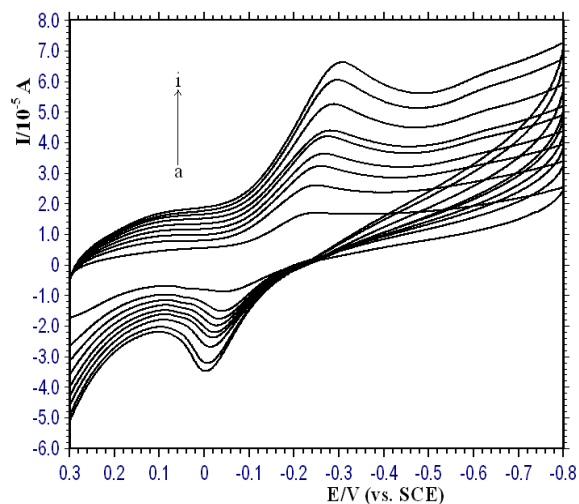

Figure 4. Cyclic voltammograms of the BMIMPF$_6$-CuS-Hb/CPE in pH 7.0 PBS with different scan rates (from a to i as 100, 150, 200, 250, 300, 350, 400, 450 and 500 mV s^{-1}).

Based on the integration of the cyclic voltammetric reduction peaks and Faraday's laws, the surface concentration of the electroactive substance (Γ^*) can be estimated by the following formula: $Q = nFA\Gamma^*$, where Q is total amount of charge passed through the electrode for the reduction reaction, A is the area of CPE, n is the number of electrons transferred in the rate determining reaction and F is the Faraday's constant. The charge values (Q) were nearly constant at different scan rates and the average value of Γ^* was calculated as 7.1×10^{-10} mol cm^{-2}, which was larger than the theoretical monolayer coverage of 2.0×10^{-11} mol cm^{-2}. The results indicated that most of the Hb molecules entrapped in the composite film participated in the electron-transfer process, which maybe due to the three dimensional structure of CuS nanosphere present on the electrode surface that was suitable for the electron transfer of Hb.

As shown in Figure 4 the redox peak potentials were also shifted gradually with the increase of the scan rate. According to Laviron's method [30],

$$E_{pc} = E^{0'} - \frac{RT}{\alpha nF} \ln \upsilon$$

(1)

$$E_{pa} = E^{0'} + \frac{RT}{(1-\alpha)nF} \ln \upsilon$$

(2)

$$\log k_s = \alpha \log(1-\alpha) + (1-\alpha)\log \alpha - \log(\frac{2.3RT}{nF\upsilon}) - \alpha(1-\alpha)\frac{nF\Delta E_p}{2.3RT}$$

(3)

where α is the charge transfer coefficient, n is the number of electrons transferred in the rate determining reaction, k_s is the electron transfer rate constant, υ is the scan rate, $E^{0'}$ is the formal potential, F is the Faraday's constant, R is the gas constant, T is the absolute temperature and ΔE_p is the peak-to-peak separation.

Based on the above equations the electrochemical parameters were further calculated by exploring the relationship of the redox peak potentials with the $\ln \upsilon$. Two linear regression equations were got as $E_{pa}(V) = 0.0393 \ln \upsilon + 0.0243$ ($\gamma = 0.998$) and $E_{pc}(V) = -0.0463 \ln \upsilon - 0.333$ ($\gamma = 0.995$), respectively. According to the equation (1) and (2) the charge transfer coefficient (α) and the number of electrons transferred (n) were got as 0.46 and 1.18, respectively. Based on the equation (3) the electron transfer rate constant (k_s) was further calculated from the relationship of ΔE_p with $\ln \upsilon$ and the result was got as 0.14 s^{-1}.

Electrocatalysis of the modified electrode

Generally speaking the immobilized Hb molecule on the electrode surface had good electrocatalytic ability due to its similar structure with peroxidase. So the electrocatalysis of $BMIMPF_6$-CuS-Hb/CPE to hydrogen peroxide was examined. As shown in Figure 5, the cyclic voltammograms of H_2O_2 on different electrodes in pH 7.0 PBS were recorded. When H_2O_2 was added into pH 7.0 PBS, a significant increase of the reduction peak at -0.40 V (vs. SCE) was observed with the disappearance of the oxidation peak (curve c-i), while no similar phenomena was observed on $BMIMPF_6$/CPE with the same solution. In the concentration range of 2.0 to 10.4 μM the catalytic reduction peak current increased with the H_2O_2 concentration and the linear regression equation was got as $I_p(\mu A) = 0.945C$ (μM) + 7.59 ($\gamma = 0.994$). The apparent Michaelis-Menten constant (K_M^{app}) was calculated from the electrochemical version of the Lineweaver-Burk equation [31].

$$\frac{1}{I_{ss}} = \frac{1}{I_{max}} + \frac{K_M^{app}}{I_{max}c}$$

where I$_{ss}$ is the steady current after the addition of substrate, c is the bulk concentration of the substrate, and I$_{max}$ is the maximum current measured under saturated substrate condition. The K_M^{app} is determined by analysis the slope and the intercept of the plot of the reciprocals of the reduction peak current versus H$_2$O$_2$ concentration, which is an indicative of the enzyme-substrate kinetics. The small value of K_M^{app} means the higher enzymatic activity of immobilized redox protein. Based on this equation the K_M^{app} value was calculated as 2.22 μM.
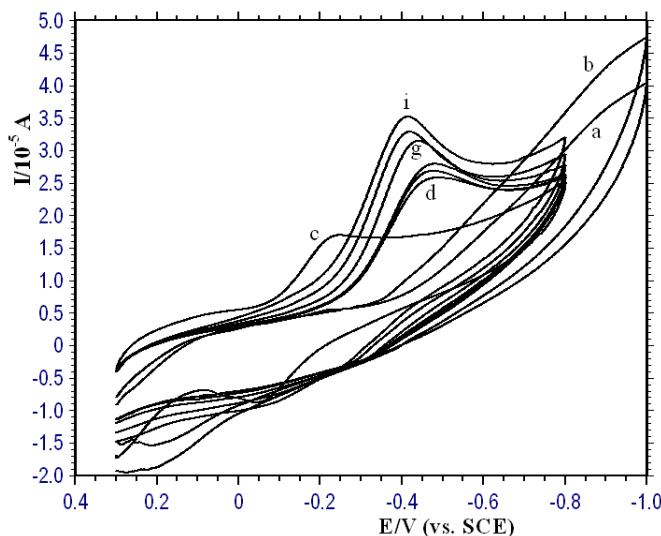

Figure 5. Cyclic voltammograms of (a) BMIMPF$_6$/CPE in pH 7.0 PBS, (b) BMIMPF$_6$/CPE in pH 7.0 PBS containing 6.0 μM H$_2$O$_2$, and BMIMPF$_6$-CuS-Hb/CPE in pH 7.0 PBS containing 0, 4.0, 6.0, 7.0, 8.6, 9.6, 10.4 μM H$_2$O$_2$ (curve c-i), respectively, at the scan rate of 100 mV s^{-1}.

Stability of the BMIMPF$_6$-CuS-Hb/CPE

When the BMIMPF$_6$-CuS-Hb/CPE was not in use, it was stored at 4 ºC in a refrigerator. After one-week storage, no obvious decrease of the redox peak response was observed. The decrease of reduction peak currents was within 3.2% after 28 days storage periods, indicating that the BMIMPF$_6$-CuS-Hb/CPE was very stable. The results demonstrated that BMIMPF$_6$ and CuS nanosphere composite was a suitable matrix to immobilize Hb and retain its activity. The reproducibility of BMIMPF$_6$-CuS-Hb/CPE was estimated from the cyclic voltammetric response for five modified electrodes prepared individually. The results revealed that the electrode had satisfactory reproducibility with its relative standard deviation (RSD) as 4.01%.

CONCLUSIONS

In this paper a stable BMIMPF$_6$-CuS-Hb biocomposite film modified CPE was prepared. The direct electrochemistry of entrapped Hb was achieved and electrochemical behaviors of the modified electrodes were investigated. A pair of well-defined redox peak appeared on the Hb modified electrode, which was attributed to the electrochemical reaction of heme Fe(III)/Fe(II)

redox couples in the Hb molecules. The modified electrode showed good electrocatalytic ability to the reduction of H_2O_2 with the reduction peak current increased in the concentration of 2.0 to 10.4 μM. So the modified electrode had the potential application for the fabrication of third generation biosensors.

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