Direct Electrochemistry and Electrocatalysis of Myoglobin with CoMoO₄ Nanorods Modified Carbon Ionic Liquid Electrode

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By using ionic liquid 1-hexylpyridinium hexafluorophosphate (HPPF₆) based carbon ionic liquid electrode (CILE) as the substrate electrode, a CoMoO₄ nanorods and myoglobin (Mb) composite was casted on the surface of CILE with chitosan (CTS) as the film forming material to obtain the modified electrode (CTS/CoMoO₄-Mb/CILE). Spectroscopic results indicated that Mb retained its native structures without any conformational changes after mixed with CoMoO₄ nanorods and CTS. Electrochemical behaviors of Mb on the electrode were carefully investigated by cyclic voltammetry with a pair of well-defined redox peaks from the heme Fe(III)/Fe(II) redox center of Mb appeared, which indicated that direct electron transfer between Mb and CILE was realized. Electrochemical parameters such as the electron transfer number (n), charge transfer coefficient (α) and electron transfer rate constant (k) were estimated by cyclic voltammetry with the results as 1.09, 0.53 and 1.16 s⁻¹, respectively. The Mb modified electrode showed good electrocatalytic ability toward the reduction of trichloroacetic acid in the concentration range from 0.1 to 32.0 mmol L⁻¹ with the detection limit as 0.036 mmol L⁻¹ (3σ), and the reduction of H₂O₂ in the concentration range from 0.12 to 397.0 μmol L⁻¹ with the detection limit as 0.0426 μmol L⁻¹ (3σ).

Key Words: Carbon ionic liquid electrode, Electrochemistry, CoMoO₄ nanorods, Myoglobin

Introduction

Direct electrochemistry of redox proteins and electrode is currently attracting widespread attentions due to its importance to understand the structure and function of proteins. The research can also provide the basic information for the development of electrochemical biosensors. Myoglobin (Mb) is an important oxygen transporting protein in the biological system, which is consisted of a polypeptide chain and a heme cofactor. However, due to the deep burying of electroactive center in the complicated structure of protein and the denaturation of proteins or the unfavorable orientation on the electrode interface, direct electron transfer of proteins on the bare electrode is difficult to be realized. Great efforts have been made to increase the electron transfer kinetics of proteins with electrodes by using mediators or promoters such as polymer films, clay, nanoparticles and biomembranes. By using these functionalized materials direct electrochemistry of proteins could be greatly enhanced on the modified electrodes with a pair of redox peaks appeared.

With the development of nanotechnology different kinds of nanoparticles had been applied to direct electrochemistry of protein. Nanostructure materials have exhibited unique properties such as large surface area, exceptional chemical stability, tunable porosity, good electrical conductivity, high mechanical strength, biocompatibility and catalytic activities. The presence of nanomaterials on the electrode can provide a novel way to enhance electron transfer rate between proteins and electrode due to the quantum size effect and surface effect. Different kinds of nanoparticles such as carbon nanotube (CNT), graphene, metal, semiconductor with various morphologies had been used in the protein electrochemistry. For example, Wang et al. investigated the direct electrochemistry and electrocatalysis of Mb adsorbed in gold nanoshells. Ruan et al. studied the direct electrochemistry and electrocatalysis of Mb based on graphene-ionic liquid (IL)-chitosan bionanocomposites. Sun et al. prepared graphene-TiO₂-IL nanocomposite film modified electrode and applied it to investigate the direct electrochemistry of hemoglobin (Hb). Ma et al. developed a biocompatible nano-platform for immobilization of Hb based on ZrO₂ nanotubes-IL for the electrochemical sensing of NaNO₂. But there are seldom reports about the application of molybdate compounds in the field of protein electrochemistry. Metal molybdates are important inorganic materials with many applications in photoluminescence, electrochemistry and catalyst. As for CoMoO₄, there are three basic types existing under atmospheric pressure, that is, the low temperature α-CoMoO₄, the high temperature β-CoMoO₄ and the hydrate CoMoO₄·nH₂O. Kichambre et al. applied CoMoO₄ as a photo anode in the polycrystalline pellet form for photovoltaic electrochemical cell. Rodriguez et al. investigated the electronic properties and phase transformations of CoMoO₄ and NiMoO₄ by X-ray absorption near-edge spectroscopy and XRD studies. Pandey et al. studied the structural, optical, electrical and photovoltaic electrochemical properties of CoMoO₄ thin film. Peng et al. proposed a large scale synthesis method of nanostruc-
tured CoMoO$_4$ by precipitation strategy. By decreasing the dimensionality, nanostructured CoMoO$_4$ exhibits many novel properties such as high surface area, optical transparency, good antibacterial activities, good biocompatibility, relatively good conductivity, which are of great importance in heterogeneous catalysis and industrial application. Ding et al. synthesized molybdate hydrates nano/microcrystals by a hydrothermal approach and investigated the magnetic, photocatalytic and electrochemical properties. Pei et al. prepared nanostructured CoMoO$_4$ catalysts supported on mesoporous silica SBA-15 with different loadings and investigated the catalytic activity of these catalysts in the oxidative dehydrogenation of propane. Xiao et al. synthesized a one-dimensional CoMoO$_4$ nanorods by hydrothermal methods and investigated the lithium storage capability. Mai et al. fabricated asymmetric supercapacitor based on hierarchical MnMoO$_4$/CoMoO$_4$ heterostructured nanowires, which showed a specific capacitance and good reversibility. Due to the specific electronic properties, CoMoO$_4$ nanomaterials have the potential application to direct electrochemistry of redox proteins.

In recent years carbon ionic liquid electrode (CILE) has been widely used in the field of electrochemical sensors. CILE was prepared by using IL as the binder and the modifier in the traditional carbon paste electrode (CPE). ILs are compounds consisted entirely of ions that exist in liquid state around room temperature, which exhibit the properties such as extraordinarily high chemical and thermal stability, good conductivity, wide electrochemical windows and good dissolving capability. Due to the present of IL in CPE, CILE has been proven to exhibit excellent performances such as resistivity towards electrode fouling, high rates of electron transfer and the inherent catalytic activity. It has been reported that CILE combines the advantages of carbon electrode such as glassy carbon electrode (GCE), carbon ceramic electrode and pyrolytic graphite electrode. So CILE has been used as the working electrode in the field of electrochemical sensors. Maleki et al. made a CILE with nocylypyridinium hexafluorophosphate and carbon powder to investigate the electrochemical behaviors of different electroactive compounds. Sun et al. fabricated an N-butylpyridinium hexafluorophosphate based CILE for the electrochemical applications. Safavi et al. fabricated a glucose sensor based on nanoscale nickel hydroxide modified CILE.

In this work, CoMoO$_4$ nanorods were synthesized and used for the investigation on the direct electrochemistry of Mb with CILE as the substrate electrode. Mb modified electrode was fabricated with the addition of CoMoO$_4$ nanorods and chitosan (CTS) was used as the film to fix the composite on the electrode surface, which could increase the stability of the modified electrode. Direct electrochemistry of Mb was realized and enhanced with a pair of well-defined redox peaks appeared, which could be attributed to the specific properties of CoMoO$_4$ nanorods. Electrochemical behaviors of Mb were carefully investigated with the electrochemical parameters calculated. The modified electrode exhibited excellent catalytic activity to the electrochemical reduction of trichloroacetic acid (TCA) and hydrogen peroxide ($H_2O_2$) with wider linear range and lower detection limit.

**Experimental**

**Reagents.** Myoglobin (Mb, MW. 17800, Sigma), 1-hexyl-pyridinium hexafluorophosphate (HPPF$_6$, Lanzhou Greenchem ILS. LICP. CAS., China), chitosan (CTS, minimum 95% deacetylated, Dalian Xindie Chemical Reagents Ltd. Co., China), graphite powder (average particle size 30 μm, Shanghai Colloid Chemical Plant, China), trichloroacetic acid (TCA, Tianjin Kemio Chemical Ltd. Co., China) and hydrogen peroxide ($H_2O_2$, Tianjin Bodi Chemical Holding Ltd. Co., China) were used as received. CoMoO$_4$ nanorods (nano-CoMoO$_4$) were synthesized based on the reported procedure. $0.1 \text{ mol L}^{-1}$ phosphate buffer solutions (PBS) with various pH values were prepared and used as the supporting electrolyte. All the other chemicals were of analytical reagent grade and doubly distilled water was used to prepare all solutions.

**Apparatus.** All the electrochemical measurements were executed on a CHI 750 B electrochemical workstation (Shanghai CH Instrument, China) with a conventional three-electrode system, which was composed of a modified CILE as working electrode, a saturated calomel electrode (SCE) as reference electrode and a platinum wire as counter electrode. UV-Visible absorption spectrum and FT-IR spectrum were operated with a Cary 50 probe spectrophotometer (Varian Company, Australia) and a Tensor 27 FT-IR spectrophotometer (Bruker, Germany), respectively. Scanning electron microscopy (SEM) was conducted on a JSM-6700FE scanning electron microscope (Japan Electron Company, Japan).

**Electrode Preparation.** CILE was prepared with the following procedure: 0.8 g of HPPF$_6$ and 1.6 g of graphite powder were ground carefully in a mortar to get an IL modified carbon paste. Then a portion of homogeneous paste was packed firmly into a glass tube ($\Phi = 4.2 \text{ mm}$) with the electrical contact established through a copper wire to the end of the paste. Before use the surface of CILE was polished on a weighing paper to get a mirror-like interface for the further modification.

The modifier was prepared by mixing Mb and nano-CoMoO$_4$ homogeneously to obtain a suspension solution with the final concentrations of 15.0 mg mL$^{-1}$ and 0.3 mg mL$^{-1}$, respectively. Then 8 μL of mixture was evenly spread onto the CILE surface and the electrode was left in the air to allow the water evaporated gradually. Finally, 5.0 μL of 1.0 mg mL$^{-1}$ CTS (in 1.0% HAC) solution was dropped on the electrode surface and dried to get a uniform film. The fabricated electrode was denoted as CTS/CoMoO$_4$-Mb/CILE and kept at 4°C refrigerator when not use. Other modified electrodes such as CTS/CILE, CTS/Mb/CILE etc. were fabricated with the similar procedures for comparison.

**Electrochemical Measurement.** The three-electrode system was immersed in a 10 mL cell containing 0.1 mol L$^{-1}$ pH 3.0 PBS and scanned in the potential range from 0.2 to -0.6 V.
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(vs SCE) at the scan rate of 100 mV s$^{-1}$. Before the measurement the buffer solutions were deoxygenated by bubbling high pure nitrogen thoroughly for 30 min and the nitrogen atmosphere environment was kept in the electrochemical cell during the procedure.

Results and Discussion

SEM Images. SEM was used to record the image of the synthesized CoMoO$_4$ nanomaterial with the results shown in Figure 1 and the inset was the enlarged magnitude of the image. It can be seen that CoMoO$_4$ appeared as rodlike nanostructures with the average diameter of 200 nm. Also some of the nanorods aggregated together to form bundles like structure. The disorder distribution of CoMoO$_4$ nanorods can form a porous structure on the electrode surface with the increase of the effective area, which is suitable for the immobilization of Mb molecules.

FT-IR and UV-Vis Absorption Spectroscopy. FT-IR spectroscopy is a sensitive tool for studying the secondary structure of heme proteins. The characteristic amide I (1700-1600 cm$^{-1}$) and amide II (1620-1500 cm$^{-1}$) bands of proteins can provide the detailed information on the secondary structure of polypeptide chain. The amide I band is caused by the C=O stretching vibration of peptide linkages in the backbone of the protein, and the amide II band is assigned to a combination of N-H bending and C-N stretching vibrations. 

FT-IR spectroscopy was recorded with the results shown in Figure 2(A). It can be seen that the amide I and II bands of native Mb were located at 1651 and 1528 cm$^{-1}$ (curve a). While the mixture of CTS, CoMoO$_4$ and Mb also gave the similar positions with the amide I and II bands at 1649 and 1525 cm$^{-1}$ (curve b). The almost same position of the wavenumber in FT-IR spectra indicated that Mb still retained its original structure after immobilized with CTS and CoMoO$_4$ nanorods.

The secondary structure of proteins in the composite can be further confirmed by UV-Vis absorption spectroscopy. In UV-Vis absorption spectrum the Soret absorption band from the four iron heme groups of heme proteins is sensitive to the variation of the microenvironment around the heme group and the band shift may provide the information about the denaturation on the tertiary structure in heme proteins. 

Figure 2(B) presented the typical UV-Vis absorption spectroscopy of Mb in the composite material. It can be seen that the native Mb gave a Soret absorption band at 409 nm in pH 3.0 PBS (curve a). While the mixture solution of CTS-CoMoO$_4$-Mb also had the Soret band at 409 nm without changes (curve b), suggested that the secondary structure of proteins did not change after mixing with CTS and CoMoO$_4$ nanorods. CTS is a biodegradable and nontoxic natural biopolymer originated from the exoskeleton of crustaceans, which has been widely reported as an immobilization matrix for the preparation of biosensor and bioreactor with excellent film-forming ability.

So the results indicated that CoMoO$_4$ nanorods were also biocompatible nanomaterials without the change of Mb structure.

Direct Electrochemistry of Mb Modified Electrode. Cyclic voltammograms of different modified electrodes in pH 3.0 PBS at the scan rate of 100 mV s$^{-1}$ were recorded and demonstrated in Figure 3. It was observed that no voltammetric responses appeared on both CILE (curve a) and CTS/CILE (curve b), indicating no electroactive substance present on the electrode. On CTS/Mb/CILE (curve c) a pair of unsymmetric redox peaks appeared on cyclic voltammogram with the cathodic peak current (Ipc) as 6.709 $\mu$A and the anodic peak current (Ipa) as 6.057 $\mu$A. The redox peak potential was got with the values of Epc as $-0.284$ V and Epa as $-0.171$ V, and the peak-to-peak separation ($\Delta E_p$) as 113 mV. The results indicated that direct electron transfer between Mb and CILE was realized with slow electron transfer.

Figure 1. SEM images of CoMoO$_4$ nanorods. Inset is the high magnification of SEM image.

Figure 2. (A) FT-IR spectra of (a) Mb film; (b) CTS/CoMoO$_4$-Mb composite film; (B) UV-Vis absorption spectra of native Mb (curve a) and CTS-CoMoO$_4$-Mb mixture (curve b) solution with pH 3.0 PBS, respectively.
indicating the presence of CoMoO$_4$ nanorods enhanced the electron transfer process of Mb in the composite film on the electrode surface. The charge value ($Q$) was nearly constant at different scan rates and the average value of $\Gamma^*$ was obtained as $7.13 \times 10^{-4}$ mol cm$^{-2}$. While the theoretical monolayer concentration of protein on the electrode surface was $2.0 \times 10^{-15}$ mol cm$^{-2}$, so multilayers of Mb on the electrode surface took place the electrochemical reaction. Also the total surface concentration of Mb in the composite film on the electrode surface was calculated as $5.36 \times 10^{-5}$ mol cm$^{-2}$, then 13.28% of the immobilized Mb molecules took part in the reaction. The results could be attributed to the presence of CoMoO$_4$ nanorods resulted in a porous structure with higher surface area. The interaction of CoMoO$_4$ nanorods with Mb also happened in the film, then the several layers of Mb molecules near the electrode surface underwent the electron transfer with the help of CoMoO$_4$ nanorods.

From Figure 4 it can also be seen that the redox peak potentials moved gradually with the increase of scan rate. The oxidation peak shifted to the positive direction and the reduction peak shifted to the negative direction with the peak-to-peak separation ($\Delta Ep$) increased, indicating a quasi-reversible process. The relationship of redox peak potentials and the natural logarithm of scan rate ($\log \nu$) were calculated, which was linearly dependant in the range from 80 to 800 mV s$^{-1}$. The results could be attributed to the presence of CoMoO$_4$ nanorods resulted in a porous structure with higher surface area. The interaction of CoMoO$_4$ nanorods with Mb also happened in the film, then the several layers of Mb molecules near the electrode surface underwent the electron transfer with the help of CoMoO$_4$ nanorods.

$$Epc = E^\circ - \frac{RT}{nF} \ln \nu$$

$$Epa = E^\circ + \frac{RT}{(1-\alpha)nF} \ln \nu$$

$$\log k = a \log(1-\alpha) + (1-\alpha) \log \nu + \frac{RT}{nF} \frac{1-\alpha}{2.3RT} E \bar{\Delta} p$$
where $\alpha$ is the electron transfer coefficient, $n$ is the number of electron transferred, $v$ is the scan rate, and $E^o$ is the formal potential, $k_r$ is the electron transfer rate constant and $\Delta E_p$ is the peak-to-peak potential separation, $R$, $T$ and $F$ have their conventional meanings. Based on these equations the value of $n$ was estimated as 1.09, suggesting that totally one electron was involved in the electrode reaction. The values of $\alpha$ and $k_r$ were further calculated as 0.53 and 1.16 s$^{-1}$. The value of $k_r$ is bigger than that of 0.34 s$^{-1}$ on Mb/NiO/ GCE$^{35}$ and 0.41 s$^{-1}$ on Mb-HSG-SN-CNTs/GCE$^{39}$ indicating that CoMoO$_x$ nanorod acted as an excellent promoter to the direct electron transfer between Mb and the electrode.

It is well-known that most of the heme proteins exhibit a pH-dependent conformational equilibrium and the pH value of the buffer solution influences the electrochemical reaction of the heme proteins. The effect of buffer pH on the response of CTS/CoMoO$_x$-Mb/CILE was investigated in the pH range from 1.0 to 7.0. The increase of buffer pH led to a negative shift of both reduction and oxidation peak potentials, which indicated that protons involved in the electrode reaction. The maximum redox peak currents were obtained at pH 3.0 buffer solution, which was selected for the further investigation.

**Electrocatalysis of CTS/CoMoO$_x$-Mb/CILE.** Because of the presence of similar active center of Mb with peroxidase, Mb exhibits intrinsic peroxidase activity in the catalytic reaction. Due to the presence of Mb on the electrode surface, the modified electrodes often showed good electrocatalytic ability toward the reduction of different substrates with a large decrease of activation energy. The electrocatalytic activity of CTS/CoMoO$_x$-Mb/CILE to TCA and H$_2$O$_2$ was investigated to probe its potential applications. Figure 5 showed the cyclic voltammograms of the modified electrode with different concentrations of TCA. It can be seen that a new reduction peak appeared at $-0.258$ V (vs SCE) with the addition of TCA, and the reduction peak currents increased gradually with the disappearance of the oxidation peak current, which was a typical electrocatalytic reduction process. The linear relationship of the electrocatalytic reduction peak current and TCA concentration was constructed in the range from 0.1 to 32.0 mmol L$^{-1}$ with the linear regression equation as $I_{ss} (\mu A) = 8.443C$ (mmol L$^{-1}$) + 34.30 ($n = 17$, $\gamma = 0.998$). The detection limit, which was calculated based on a signal-to-noise ratio of 3, was got as 0.036 mmol L$^{-1}$ (3$\sigma$). When the TCA concentration was more than 32.0 mmol L$^{-1}$ the catalytic reduction peak currents began to level off with a plateau appeared, which was a typical Michaelis-Menten kinetic mechanism. So the apparent Michaelis-Menten constant ($K_{Mapp}$), which gives an indication of the enzyme-substrate kinetics, can be obtained from the following Lineweaver-Burk equation:

$$\frac{1}{I_{ss}} = \frac{1}{I_{max}} + \frac{K_{Mapp}}{I_{max}}c$$

where $c$ is the bulk concentration of the substrate, $I_{ss}$ is the steady current after the addition of substrate, and $I_{max}$ is the maximum current measured under saturated substrate condition. Then the $K_{Mapp}$ value was calculated as 0.547 mmol L$^{-1}$ with the above equation. So the Mb molecules immobilized on the electrode surface exhibited a high affinity for TCA. In order to compare the electrochemical performance of Mb modified electrodes, the electrochemical data were summarized in Table 1. It can be seen that the CTS/CoMoO$_x$-Mb/CILE exhibited wider linear range and lower detection limits for TCA detection.

**Electrocatalysis of CTS/CoMoO$_x$-Mb/CILE towards the reduction of H$_2$O$_2$** was further investigated with the cyclic voltammograms shown in Figure 6. With the addition of different amounts of H$_2$O$_2$ into PBS, a new reduction peak appeared at $-0.255$ V, indicating the electrocatalytic reduction. So the electrocatalytic reduction of H$_2$O$_2$ by Mb on the modified electrode can be explained with the following

![Figure 5. Cyclic voltammograms of CTS/CoMoO$_x$-Mb/CILE in the presence of 2.0, 4.0, 6.0, 8.0, 10.0, 12.0, 16.0, 22.0, 26.0, 30.0 mmol L$^{-1}$ TCA (a-j) in pH 3.0 PBS at the scan rate of 100 mV s$^{-1}$.](Image 320x597 to 537x745)

**Table 1.** Electrochemical data of different Mb modified electrodes towards the reduction of TCA

<table>
<thead>
<tr>
<th>Modified Electrodes</th>
<th>Linear Range (mmol L$^{-1}$)</th>
<th>Detection Limit (mmol L$^{-1}$)</th>
<th>Sensitivity (µA mM$^{-1}$)</th>
<th>$K_{Mapp}$ (mmol L$^{-1}$)</th>
<th>Refs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mb/DNA/CILE</td>
<td>0.5-40.0</td>
<td>0.083</td>
<td>10.6</td>
<td>0.82</td>
<td>41</td>
</tr>
<tr>
<td>Nafion/Mb/CILE</td>
<td>0.4-12.0</td>
<td>0.2</td>
<td>–</td>
<td>4.11</td>
<td>42</td>
</tr>
<tr>
<td>Nafion-BMIMPF$_x$/Mb/CPE</td>
<td>0.2-11.0</td>
<td>0.016</td>
<td>48</td>
<td>–</td>
<td>43</td>
</tr>
<tr>
<td>Mb/ZrPNS/GCE</td>
<td>0.1-2.2</td>
<td>0.025</td>
<td>–</td>
<td>–</td>
<td>44</td>
</tr>
<tr>
<td>Nafion/Mb/MWCNTs/CILE</td>
<td>1.57-12.0</td>
<td>0.1</td>
<td>58.2</td>
<td>3.396</td>
<td>45</td>
</tr>
<tr>
<td>CTS/CoMoO$_x$/Mb</td>
<td>0.1-32.0</td>
<td>0.036</td>
<td>8.443</td>
<td>0.547</td>
<td>This work</td>
</tr>
</tbody>
</table>

BMIMPF$_x$: 1-Butyl-3-methylimidazolium hexafluorophosphate; ZrPNS: zirconium phosphate nanosheets; MWCNTs: multi-walled carbon nanotubes.
ZrPNS: zirconium phosphate nanosheets. nanotube; HMS: hexagonal mesoporous silica; IL: ionic liquid; HSG: hybrid silica sol-gel; SN-CNTs: Ag nanoparticle doped carbon nano-tubs; PDDA: poly(diallyldimethylammonium); PGE: pyrolytic graphite electrode; GCE: glassy carbon electrode; SG: sol-gel; MWCNT: multi-walled carbon nanotube; MB: myoglobin; TCA: 2,3,5-trichloroacetanilide; CILE: carbon impregnated titanium electrode; CoMoO$_4$: cobalt-doped molybdenum oxide; CTS/CoMoO$_4$: carbon thin film-coated CoMoO$_4$; 3,4-DHP: 3,4-dihydroxyphenylalanine; H$_2$O$_2$: hydrogen peroxide. 

Table 2. Electrochemical data of different Mb modified electrodes towards the detection of H$_2$O$_2$.

<table>
<thead>
<tr>
<th>Modified Electrodes</th>
<th>Linear Range (mmol L$^{-1}$)</th>
<th>Detection Limit (μmol L$^{-1}$)</th>
<th>Sensitivity (μA mM$^{-1}$)</th>
<th>$K_{M}$ (mmol L$^{-1}$)</th>
<th>Refs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mb-HSG-SN-CNTs/GCE</td>
<td>2.0-1200</td>
<td>0.36</td>
<td>–</td>
<td>1.62</td>
<td>39</td>
</tr>
<tr>
<td>Mb/ZrPNS/GCE</td>
<td>0.8-12.8</td>
<td>0.14</td>
<td>–</td>
<td>0.034</td>
<td>44</td>
</tr>
<tr>
<td>{SG-Al$_2$O$<em>3$/Mb$</em>{30}$/PGE}</td>
<td>1.0-125</td>
<td>0.6</td>
<td>0.265</td>
<td>–</td>
<td>46</td>
</tr>
<tr>
<td>Mb/ZrO$_2$/MWCNT/GCE</td>
<td>1.0-116.0</td>
<td>0.53</td>
<td>17</td>
<td>0.085</td>
<td>47</td>
</tr>
<tr>
<td>Mb/HMS/GCE</td>
<td>4.0-124</td>
<td>0.062</td>
<td>424</td>
<td>0.065 ± 0.005</td>
<td>48</td>
</tr>
<tr>
<td>Mb/clay-IL/GCE</td>
<td>3.9-259</td>
<td>0.733</td>
<td>–</td>
<td>0.0173</td>
<td>49</td>
</tr>
<tr>
<td>Mb-TiO$_2$/MWCNT/GCE</td>
<td>1-160</td>
<td>0.41</td>
<td>16.4</td>
<td>0.0831</td>
<td>50</td>
</tr>
<tr>
<td>PDDA/[ZrO$_2$]$_7$/Mb/PGE</td>
<td>0.02-3.0</td>
<td>0.01</td>
<td>2970</td>
<td>–</td>
<td>51</td>
</tr>
<tr>
<td>CTS/CoMoO$_4$-Mb/CILE</td>
<td>0.02-3.0</td>
<td>0.01</td>
<td>2970</td>
<td>–</td>
<td>51</td>
</tr>
</tbody>
</table>

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Motor, Mb Fe(III) + H$^+$ + e$^-$ $\rightarrow$ Mb heme Fe(II)

The reduction peak current increased linearly with H$_2$O$_2$ concentration in the range from 0.12 to 397.0 μmol L$^{-1}$ with the linear regression equation as $\ln(\mu A) = 0.0686C$ (μmol L$^{-1}$) + 16.62 ($n = 19$, $\gamma = 0.998$) and the detection limit was calculated as 0.0426 μmol L$^{-1}$ (3σ).

Based on the same method the apparent Michaelis-Menten constant ($K_M$) was further calculated as 0.016 mmol L$^{-1}$. A comparison of the results for the determination of H$_2$O$_2$ with different modified electrodes was also summarized with the results listed in Table 2. Compared with other H$_2$O$_2$ biosensors, the fabricated CTS/CoMoO$_4$-Mb/CILE had wider linear range, lower detection limit, higher sensitivity and smaller $K_M$.

Interference. The influence of some possible interferents on the voltammetric responses of 4.0 μmol/L H$_2$O$_2$ was investigated by using the tolerable limit with the relative deviation of ±5%. No obvious interferences could be observed with 1000 fold concentrations of K$^+$, NH$_4^+$, Zn$^{2+}$, Mg$^{2+}$, SO$_4^{2-}$, Cl$^-$, and 500 fold of ascorbate, uric acid, acetaminophenol. The results indicated that this modified electrode exhibited good selectivity.

Stability and Reproducibility. The stability and reproducibility of CTS/CoMoO$_4$-Mb/CILE was also studied. The electrode was stored at room temperature when not in use, it could remain 97.88% and 94.1% of its initial responses after storage of 14 days and 30 days, respectively, which indicated that the Mb modified electrode had good stability. Four modified electrodes were fabricated with the same procedure and further applied to the detection of 20.0 mmol L$^{-1}$ TCA with a relative standard deviation (RSD) value of 2.37%. The results indicated that CTS/CoMoO$_4$-Mb/CILE exhibited good stability and reproducibility in the electrochemical application.

Conclusion

CoMoO$_4$ nanorods were incorporated with Mb and further modified on the CILE surface to get the Mb modified electrode. The presence of CoMoO$_4$ nanorods acted as an efficient promoter to accelerate the direct electron transfer of Mb with CILE, which may be due to the specific properties of nanostructured CoMoO$_4$. A pair of well-defined redox peaks appeared on CTS/CoMoO$_4$-Mb/CILE, which was attributed to the direct electron transfer of Fe(III)/Fe(II) in the redox protein. The electrocatalysis of Mb in the modified electrode to the reduction of TCA and H$_2$O$_2$ was further investigated with wider linear range and lower detection limit. The CTS/CoMoO$_4$-Mb/CILE electrode exhibited the advantages such as long-term stability, high electrocatalytic ability, good reproducibility and simply preparation procedure. The results indicated the CoMoO$_4$ nanorods had potential applications in the field of the electrochemical enzyme biosensor.

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