SHORT COMMUNICATION

Yuzhong Zhang · Guiying Jin · Wangxing Cheng

Electrochemical behavior of electrodeposited film derived from rhein and its activity towards myoglobin reduction

Abstract The stable electroactive thin film of rhein has been investigated by cyclic voltammetry and electrochemical impedance spectroscopy. Electrochemical impedance spectroscopy of the electrodeposited film derived from rhein indicated the electrode reaction was kinetically controlled in the region of higher frequency, the charge transfer resistance was 2.6×10^3 Ω cm^2 and capacitance value was 13.2 μF cm^2 . The electrodeposited film derived from rhein exhibited a good electrocatalytic activity for myoglobin (Mb) reduction. In 0.30 mol dm^{-3} H$_2$SO$_4$solution, the catalysis currents were proportional to the concentrations of Mb over the range of 1.5×10^{-7}–1.3×10^{-5} mol dm^{-3}. The detection limit is 1.0×10^{-7} mol dm^{-3} ($S/N=3$). The relative standard deviation is 4.8% for eight successive determinations of 5.0×10^{-7} mol dm^{-3} Mb.

Keywords Myoglobin · Rhein · Electrocatalysis · Chemically modified electrode · Electrochemical impedance spectroscopy

Introduction

The physiological function of Myoglobin (Mb) is to store dioxygen and to increase its diffusion rate in the cell. Although Mb has no physiological function as an electron carrier, it will undergo oxidation and reduction. Because of its three-dimensional structure and inaccessibility to the electroactive center, the transfer rate of heterogeneous electron for Mb is rather slow at conventional electrode, so facilitation of electron transfer between the protein Mb and electrodes is very

challenging task. One way to overcome the problem is to build protein film, which incorporated redox proteins in. It can reside in bilayer biomembrane-like microenvironment and improve its electrochemical property [1]. A series of studies on electrochemistry of Mb in stable films have been reported [2–7], such as lipid films of dimyristoyl phosphatidylcholine (DDAB) [2, 3], composite of didodecyldimethylammonium bromide (DDAB)-clay [4], and phosphatidylcholines (PC) film [5]. Another way is to modify the electrode surface with mediator, which can undergo fairly fast redox reaction with Mb. The mediated electron transfer enables efficient electrocatalytic reaction of Mb. Up to now many compounds have been used as mediator to enable efficient electrocatalytic reaction of Mb [8–11], such as methylene blue [8, 9], azure I [8], toluidine blue [10]. Although there are many studies on the electrochemical reduction of Mb using various modified electrodes, to our knowledge, there are no reports about the electrochemical catalysis reduction of Mb using a rhein-modified glassy carbon electrode.

We have investigated the electrodeposition of rhein (4.5-dixydroxyanthraquinone-2-carboxylic acid) (Fig. 1), characterized rhein film and tested its electrocatalytic activity toword hemoglobin reduction [12]. In the present work, we investigate electrochemistry impedance spectroscopy of electrodeposited film derived from rhein, and tested its electrocatalytic activity toward Mb reduction. The results indicated that the electrodeposited film derived from rhein had a good electrochemical activity toward reduction of Mb.

Experimental

Chemicals

Myoglobin (Horse Skeletal Muscle) was purchased from ICN Biomedicals Inc and rhein was purchased from the National Institute for the Control of Pharmaceutical and Biological Products in China. Stock solution of Mb

Y. Zhang (✉) · G. Jin · W. Cheng
College of Chemistry and Materials Science,
Anhui key Laboratory of Functional Molecular Solids,
Anhui Normal University, Wuhu, 241000, China
E-mail: zhangyz65@hotmail.com
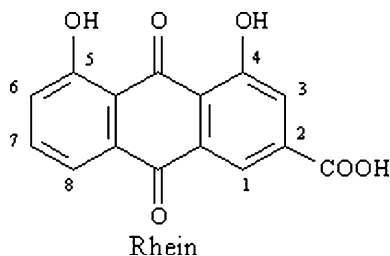Tel.: +86-553-3869303
Fax: +86-553-3869303

Fig. 1 The structure of rhein

was stored at a temperature of 4°C. All others reagents were of analytical grade. All the solutions were prepared in double distilled water.

Apparatus

A CHI660A electrochemical workstation (Chenghua Instruments Corp, Shanghai, China) was used for electrochemistry measurement. A conventional three-electrode system was employed with a bare or rhein-modified GCE (3.0 mm in diameter) as the working electrode, a platinum coil as the auxiliary electrode, and a Ag/AgCl electrode as the reference electrode. All potentials reported in this paper were referenced to Ag AgCl. A pHS–3C Acidic meter (Tianda Instruments Corp, Shanghai, China) was used to determine the pH value of solution.

Procedures

The glassy carbon electrode was polished mechanically with emery paper and alumina paste (0.3μm) to a mirror-like finish, sonicated, rinsed with water and further cleaned in 1:1 nitric acid, acetone and double distilled water. Prior to use, the electrode was treated electrochemically by a continuous cyclic potential sweep from –1.2 V to +1.4 V at a scan rate of 50 mV/s in 0.50 mol dm^{-3} H_2SO_4 solution until a stable voltammogram was obtained.

The activated electrode was modified as follows. It was placed in acetate buffer solution (pH 4.5) containing 1.0×10^{-5} mol dm^{-3} rhein and was modified by cycling the potential between -0.8 V and $+0.4$ V at 50 mV/s for ten circles. After the rhein film had been formed on the electrode surface, the electrode was rinsed thoroughly with water and dipped into the H_2SO_4 solution to test its electrochemical behavior and examine the electrocatalytic activity toward Mb reduction in the potential range of -0.6 to $+0.1$ V.

All measurements were performed at the ambient temperature (ca. 18 ± 1°C). The electrochemical experiments were performed in a 10 mL electrolytic cell with 5 mL solution, from which oxygen was removed by purging highly pure nitrogen for 10 min before electrochemical experiments and a continuous flow of nitrogen was maintained during experiments.

Results and discussion

Electrochemistry behavior of the electrodeposited film derived from rhein

As described previously [12], rhein can be oxidatively electrodeposited by cyclic voltammetry on glassy carbon electrode previously activated in 0.5 mol dm^{-3} H_2SO_4 solution. The mechanism of electrodeposition appears to involve oxidation of the hydroquion, followed by nucleophilic attack by active groups present on the electrode surface. This process can be influenced by the presence of nucleophilic species in the electrolyte capable of competing with the reactive groups present at the electrode surface. As a result, the electrodeposition process and the electrochemical behavior of the modified electrodes are strongly dependent on pH of solution. The electrodeposited film derived from rhein exhibited a stable and well-defined redox wave.

The peak currents were directly proportional to the scan rates below 300 mV/s, so the electrochemical responses of electrodeposited film derived from rhein were those anticipated for a surface-confined redox. The cathodic and anodic peak currents were almost equal and the peak potential separation was 42 mV, indicating they were quasi-reversible redox peak.

Impedance spectroscopy of the electrodeposited film derived from rhein

The impedance of the electrodeposited film derived from rhein was measured using equal concentration of $K_3Fe(CN)_6$ and $K_4Fe(CN)_6$ as redox probe. The results were shown in Fig. 2. In the region of higher frequency, the electrode reaction was purely kinetically controlled, and there was a semicircle at the higher frequency in the impedance spectroscopy, of which the diameter presents
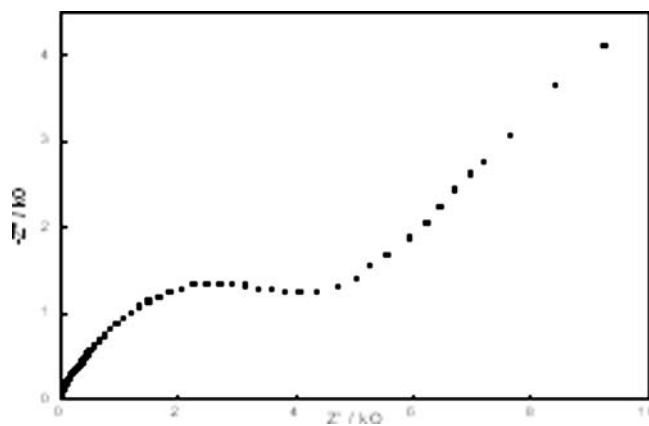

Fig. 2 The Nyquist plots for rhein-modified GCE in the presence of 1.0 mmol dm^{-3} $K_3Fe(CN)_6$ and 1.0 mmol dm^{-3} $K_4Fe(CN)_6$ in the solution of pH 7.0 Phosphate buffer solution. Potential is 5 mV; the frequency range from 1 Hz to 100 KHz

R_{ct}, the charge transfer resistance, the values of R_{ct} was 2.6×10^3 Ω cm^2, the frequency (ω_p) at the top of the semicircle in the impedance spectrum can be used to calculate the value of capacitance (C_{dl}) of the interface. According to the equation: $C_{dL} = 1/(\omega_p R_{ct})$, capacitance values 13.2 µF cm^{-2} can be obtained. It was noticed that the impedance spectra has no standard semicircle, which means that more complicated electrode reaction mechanism should be taken into account.

Electrocatalysis activity towards Mb at the electrode posited film derived from rhein

In order to test the electrocatalytic activity of the electrodeposited film derived from rhein, the cyclic voltammograms were obtained in the absence and presence of Mb. The CV curves are shown in Fig. 3. A pair of well-behaved reduction–oxidation peaks is observed in the absence of Mb (Fig. 3a). The current value of the oxidation (I_o) and the reduction peak(I_R) is -0.37 µA and 0.67 µA, respectively. The peak potential value is -0.189 V and -0.220 V, respectively. After addition of 1.0×10^{-6} mol dm^{-3} Mb, the reduction peak current (I_{cat}) was increased to 1.49 µA while the oxidation peak current decreased to -1.97µA. The ratio of reduction peak current (I_{cat}/I_R) is about 22, indicating the rhein-modified electrode has a good electrocatalytic activity towards myoglobin reduction (Fig. 3b).

The effect of H$_2$SO$_4$ concentration on the response of peak current (I_p) was investigated over the range of 0.05–0.5 mol dm^{-3}. Figure 4 shows that peak current increased with increasing H$_2$SO$_4$ concentration until it reached 0.3 mol dm^{-3}. Then peak current approached a constant when the H$_2$SO$_4$ concentration $>$ 0.3 mol dm^{-3}. So 0.3 mol dm^{-3} H$_2$SO$_4$ solution was chosen for the support electrolyte in the experiment.

Fig. 4 The relationship between peak current (Ip) and concentration of H$_2$SO$_4$

The square wave voltammetry (SWV) has not only the advantage over CV in signal to noise ratio and resolution, but it also has higher sensitivity. So it was used to determine Mb concentration. Figure 5 shows the typical SWVs obtained for a series of Mb concentration at rhein-modified glassy carbon electrode. Curve a–d in Fig. 5 show SWV recordings of various Mb concentrations. It can be seen that the peak current is 2.750, 3.500, 4.708 and 5.709 µA, respectively, with the peak potential being -0.18 V. It can be seen that the peak current increased continually with increasing Mb concentration, the background-subtracted currents were plotted against the concentration of Mb and calibration plot was linear over the range of 1.5×10^{-7}–1.3×10^{-5} mol dm^{-3}. The
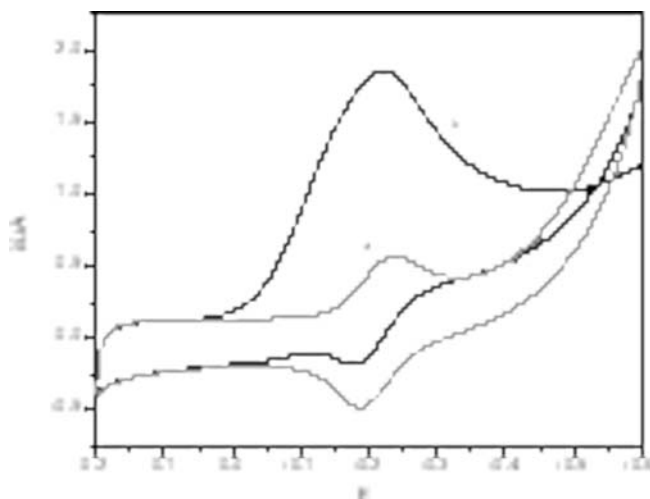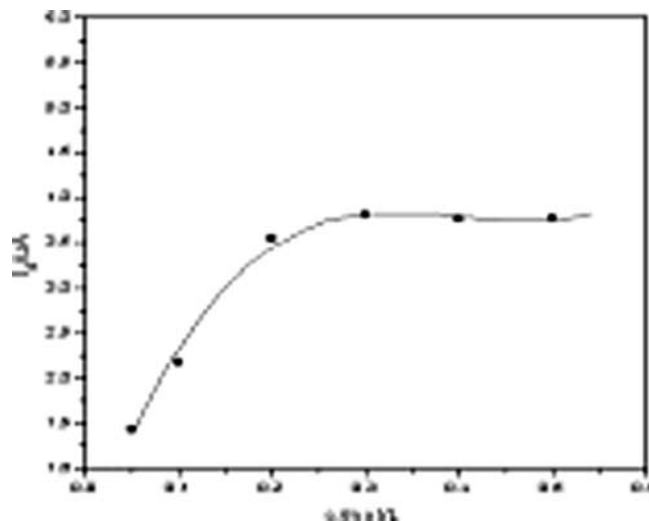

Fig. 3 Cyclic voltammograms of the electrodeposited film derived from rhein in 0.30 mol dm^{-3} H$_2$SO$_4$. **a** 0 mol dm^{-3} Mb, **b** 1.0×10^{-6} mol dm^{-3} Mb
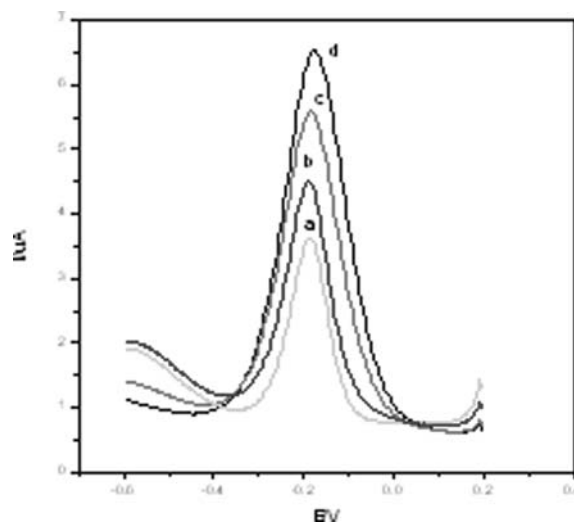

Fig. 5 Square wave voltammograms at modified electrode using various concentrations of Mb (mol dm^{-3}): **a** 0, **b** 2.0×10^{-6}, **c** 4.0×10^{-6}, **d** 6.0×10^{-6}, The experiment parameters: amplitude is 0.025 V; frequency is 15 Hz; quiet time is 2 s; scan rate is 4 mV/s; potential scan range is within –0.6 to +0.2 V

linear regression equation is ΔIp (μA) = 0.093 + 1.086×10^6 C (mol dm^{-3}), R = 0.9965. The detection limit is 1.0×10^{-7} mol dm^{-3} ($S/N = 3$). The relative standard deviation is 4.8% for eight successive determinations of 5.0×10^{-6} mol dm^{-3} Mb, indicating the rhein-modified electrode had an excellent reproducibility.

The influence of possible interfering compounds such as dopamine and ascorbic acid were examined, the experimental results showed that dopamine and ascorbic acid did not interfere with the detection of Mb in SWV. However, if Hb co-exists in the sample, it can severely interfere Mb determination.

Conclusion

The results obtained here show that the activated glassy carbon surface can be modified easily by rhein during potential recycling. The results of cyclic voltammetry show that the rhein acts as a mediator to catalyze the electroreduction of Mb in 0.30 mol dm^{-3} H$_2$SO$_4$ solu-tion. Peak currents are proportional to the concentration of Mb in the range of 1.50×10^{-7}–1.3×10^{-5} mol dm^{-3} and detection limit is 1.0×10^{-7} mol dm^{-3} ($S/N = 3$).

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