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Differential pulse anodic stripping voltammetry detection of metallothionein at bismuth film electrodes

Minli Yang [a], Zhanjun Zhang [b], Zhongbo Hu [b], Jinghong Li [a,*]

[a] *Department of Chemistry, Key Lab. of Bioorganic Phosphorus Chemistry & Chemical Biology, Tsinghua University, Beijing 100084, China*
[b] *College of Chemistry and Chemical Engineering, Graduate School of the Chinese Academy of Sciences, Beijing 100049, China*

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Abstract

As a representation of metalloproteins, metallothionein (MT), which plays important biological and environmental roles such as in the metabolism and detoxification of some metals, was detected at bismuth film electrode (BiFE) by differential pulse anodic stripping voltammetry (DPASV). In pH 2–5.5, two well-defined anodic peaks were produced and attributed to the Zn^{2+} and Cd^{2+} inherent to MT. The calibration plot of DPASV peak currents for Cd^{2+} inherent to MT versus MT concentrations showed a good linearity with a detection limit of 3.86×10^{-8} mol/L for MT. As a non-toxic excellent electrode material, BiFE shows good performance for detecting MT, and is expected to find further applications in the studies of many other metalloproteins.

Keywords: Metallothionein; Bismuth film electrode; Differential pulse anodic stripping voltammetry

1. Introduction

Metal ions play many important roles in natural proteins, from the stabilization of tertiary structure to the direction of protein folding to crucial roles in electron transfer and catalysis [1]. The detection of metal ions inherent to metalloproteins is the first step to understand metalloproteins. Metallothioneins (MTs) are a group of proteins with low molecular weight, rich cysteine residues, and high metal-binding capacity. The protein has the shape of a dumbbell and envelops the metals in two separate cluster-structured domains, one of which contains three metal atoms bound to nine cysteines and the other has four metal atoms and 11 cysteines [2]. Their important biological and environmental roles, such as in the metabolism and detoxification of some metals (Zn, Cd, Cu, Hg) [3], have attracted a great deal of interest from biochemists, electrochemists, etc. Special attention has been paid to the studies of complexing properties of MTs with heavy metal ions, with the aim of obtaining a better understanding of the biological functions of MT [3].

Due to the electroactivity of the sulphydryl groups and bound metal ions in MT [4], some electrochemical techniques [4–18] have been applied to the research of MT. It is worth noting that most of the electrochemical researches of MT were preformed at various mercury electrodes. In spite of its excellent electrochemical behavior, the use of mercury has been severely restricted and even has been completely banned by some countries because of its toxicity [19]. Therefore, it is very necessary to find a comparative alternative to mercury electrode and explore the electrochemical response of MT at the new electrode.

Wang et al. [19] firstly introduced the bismuth film electrode (BiFE) to anodic stripping voltammetry and made a series of researches of the electrochemical performance of BiFE. Since bismuth is an environmentally friendly element with very low toxicity and a widespread pharmaceutical use [20,21], BiFE has been attracting much interest from analysts [22–26]. In this work, we investigated the differential pulse anodic stripping voltammetry (DPASV) response of the Cd^{2+} and Zn^{2+} inherent to metallothionein at BiFEs, and reached a conclusion that BiFE was suitable for the electrochemical study of metalloproteins.

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[*] Corresponding author. Tel.: +86 10 62795290.
E-mail address: jhli@mail.tsinghua.edu.cn (J. Li).

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2. Experimental

2.1. Chemicals

The metallothionein from rabbit liver (M7641, containing 3% Cd and 1% Zn) was purchased from Sigma Chemical Co. (St. Louis, MO, USA). Bismuth chloride of analytical grade was obtained from Shanghai No. 2 Chemicals Factory (Shanghai, China). Acetic acid, hydrochloric acid, and sodium hydroxide were of analytical grade and purchased from Beijing Chemical Reagents Company. All solutions were prepared with deionized water produced by a Millipore system (Simplicity 185, Millipore).

2.2. Apparatus

Stripping voltammetry was performed with a CHI 440 electrochemical analyzer (CH Instruments, Austin, TX), in connection with a personal computer. A bismuth-film coated glassy carbon electrode (2 mm in diameter) served as the working electrode, with the Ag/AgCl (3 M KCl) electrode and platinum wire as the reference and counter electrodes, respectively. All potentials were given versus the Ag/AgCl (3 M KCl) electrode.

2.3. Procedure

The glassy carbon electrode (GCE) was hand-polished with a slurry of alumina and rinsed with deionized water, and then sonicated in absolute ethanol and deionized water. After being dried under a stream of N_2, the GCE was immersed in a plating solution consisting of 0.5 M HCl and 1 mM Bi^{3+} ions, which was deaerated with pure N_2 for 10 min. The ex-situ deposition of bismuth film was performed at −0.6 V for 10 min. Having been thoroughly rinsed with deionized water and dried under a stream of N_2, the electrode was immediately transferred to the solution to be analyzed.

Firstly, the background response of 0.1 M acetate solution of different pH values was detected at BiFE. The working electrode potential was held at −1.4 V for 600 s. Without any quiet time, the voltammograms were recorded between −1.4 V and −0.3 V by applying a differential pulse waveform whose parameters were as follows: increment potential of each point of 4 mV, pulse amplitude of 50 mV, pulse width of 50 ms, data sampling width of 16.7 ms, and pulse period of 200 ms. The DPASVs of MT in acetate buffer were recorded under the same parameter conditions. To avoid the presence of oxygen in the cell, the sample solution was deaerated with N_2 for 15 min prior to the electrochemical measurement and the pure N_2 was passed over the surface of the solution during the measurement step of the experiments.

3. Results and discussion

The present work adopted the following experimental parameters for the ex-situ preparation of BiFE: 1 mM Bi^{3+} ions in 0.5 M HCl, deposition potential of −0.6 V, deposition time of 10 min, without stirring. After plating, a layer of bismuth film of ca. 7.6 mC was obtained. The thickness of the bismuth film layer, presumedly calculated from Faraday's law, was 1.79×10^{-5} cm, which can be treated as the average one [22]. At such a BiFE, the DPASV responses of 0.1 M acetate solutions of different pH values were examined. It can be seen from Fig. 1 that no peak appeared in a wide potential window, which guaranteed the well-defined voltammetric responses of species to be detected.

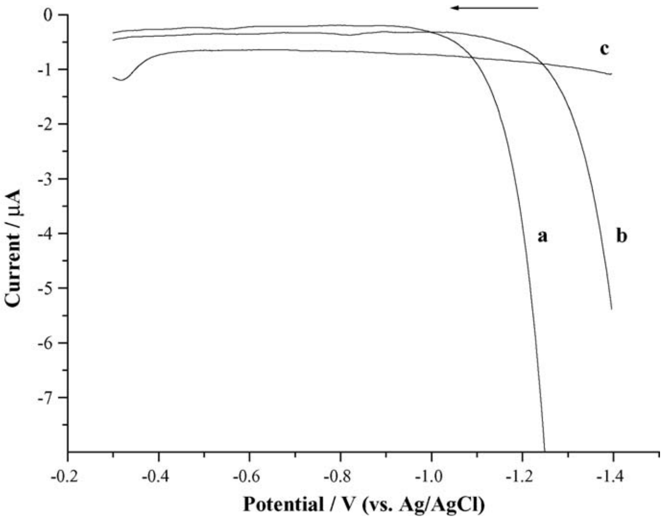

Fig. 1. DPASV responses of the preplated BiFE in 0.1 M acetate solution. Supporting solutions: (a) pH 2; (b) pH 4.5; (c) pH 7. Deposition at −1.4 V for 600 s, quiet time of 0 s, potential scope between −1.4 V and −0.3 V, increment potential of each point of 4 mV, pulse amplitude of 50 mV, pulse width of 50 ms, data sampling width of 16.7 ms, and pulse period of 200 ms.

The anodic stripping voltammetric responses of the free Cd^{2+} and Zn^{2+}were tested. Fig. 2A shows the DPASV responses of the free Cd^{2+} (2 μM) and Zn^{2+}(2 μM) in 0.1 M acetate solution (pH 4.5) at a BiFE with different deposition time of 0, 30, 60, 120, 240, 600 s. It can be observed that the anodic peak currents increased with the deposition time and the peak positions slightly shifted to more positive values. In the case of the deposition time of 600 s, two well-defined anodic stripping peaks were produced at −1.144 V and −0.784 V, which were corresponding to the free Zn^{2+} and Cd^{2+}, respectively. Also, the influence of solution pH was examined. It should be noted that, Hocevar et al. [27] studied the effect of solution pH upon the stripping voltammetric response of Cd^{2+} at in-situ BiFE showing an optimum pH range of 4.5–5.0. In their experiment, the influences included not only those on the bismuth film formation but also those on the anodic stripping voltammetric responses of the free Cd^{2+}. This work focused on the influence of solution pH on the anodic stripping voltammetric responses of the free Cd^{2+} and Zn^{2+} by ex-situ BiFE. Fig. 2B displays that the positions of current peaks corresponding to Cd^{2+} and Zn^{2+} shifted to more negative potentials with the increase of solution pH value, while the peak currents showed two different trends, a gradual decrease for Cd^{2+} peaks and a rapid increase [28] for Zn^{2+} peaks. We think that, the different changes probably resulted from proton reduction [29,30]: with the increase of pH, more Cd^{2+} was combined with OH^- resulting in the decrease of free Cd^{2+} [31]; in the case of Zn^{2+}, the negative influence of the accompanying proton reduction

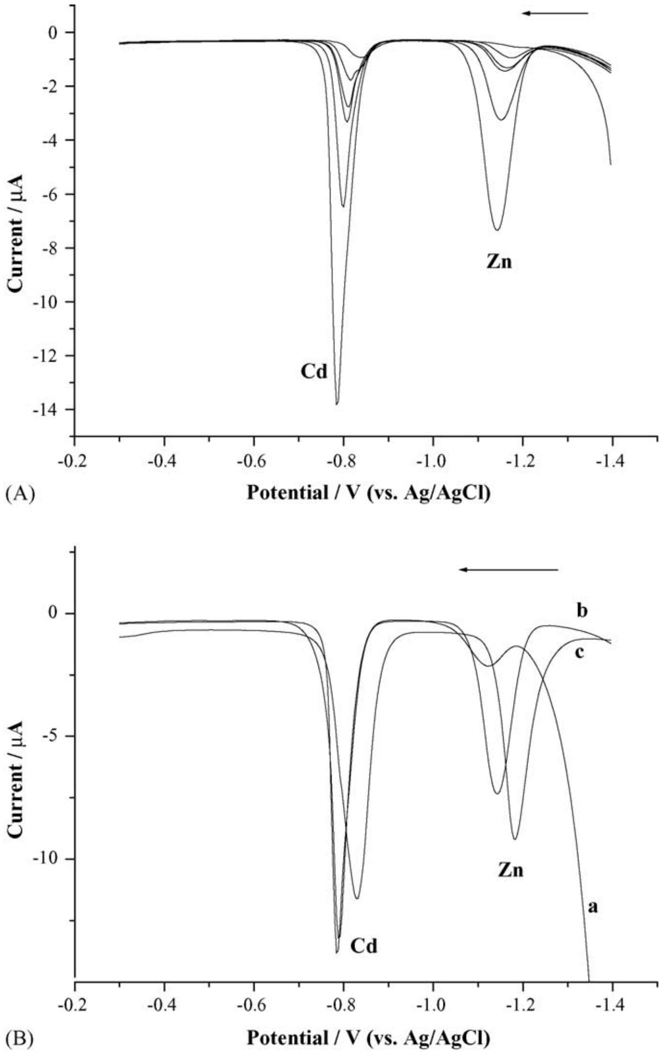

Fig. 2. (A) DPASV responses of the free Cd^{2+} (2 μM) and Zn^{2+} (2 μM) in 0.1 M acetate solution (pH 4.5) at a BiFE, with deposition time of 0, 30, 60, 120, 240, 600 s. (B) Effect of solution pH: (a) pH 2; (b) pH 4.5; (c) pH 7, deposition time of 600 s. Other experimental conditions as in Fig. 1.

became weak at high pH, which counteracted and exceeded the effect of Zn^{2+} combining with OH^-.

MTs are rich in heavy metals (Cd and Zn), whose high metal binding capacity to molecular size ratio makes them interesting for a number of reasons [4]. Fig. 3A shows the DPASV responses of 1 μM MT in acetate solutions of different pH values. When pH = 2, 3.5, 4.5, 5.5, the peaks labeled Cd(MT) and the ones labeled Zn(MT) were produced within a potential scope from −0.732 V to −0.764 V and a scope from −1.076 V to −1.116 V, respectively. The closeness of peak potentials suggested that same anodic reaction should occur at the BiFE surface. According to the paper by Ruiz and Rodriguez [5], at acidic pH (i.e. pH < 3) the cations initially bound to the thiol groups are removed, that is to say, freely exist the Cd^{2+} and Zn^{2+}. Thereby, we deduce that, the anodic peaks in Fig. 3A could be attributed to the stripping of the Cd^{2+} and Zn^{2+} from BiFE.

$$\mathrm{Cd(Bi)} \rightarrow \mathrm{Cd}^{2+} + 2e^-$$

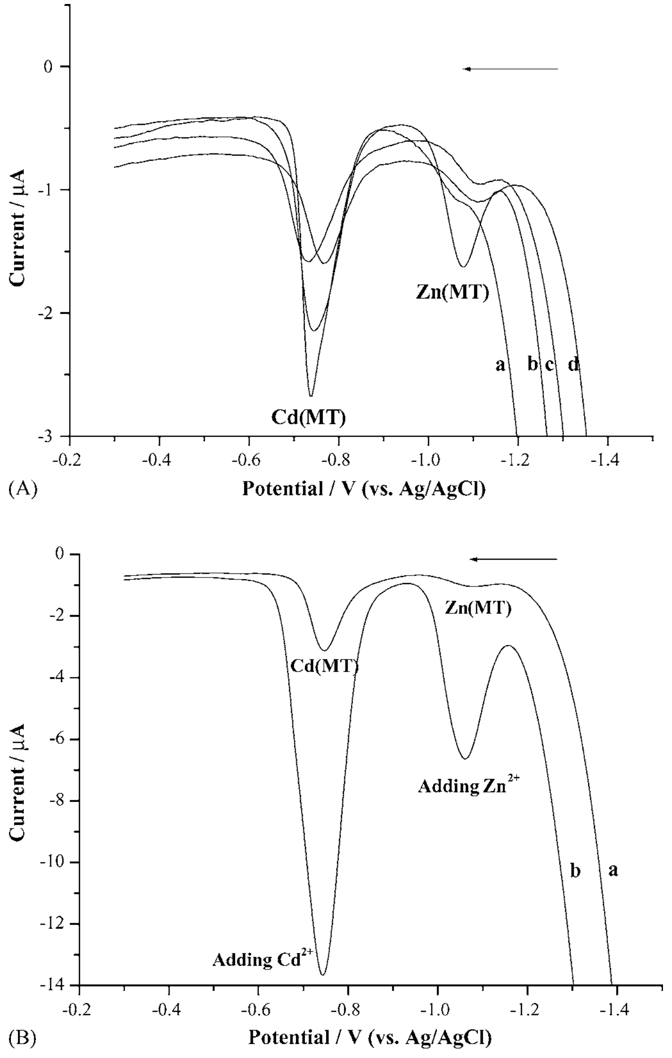

Fig. 3. (A) DPASV responses of 1 μM MT in acetate solutions at BiFE: (a) pH 2.0; (b) pH 3.5; (c) pH 4.5; (d) pH 5.5, deposition time of 600 s. (B) DPASV responses of 1 μM MT in acetate solution of pH 4.0 whithout (a) and with (b) the addition of 0.5 μM Cd^{2+} and 0.5 μM Zn^{2+}, deposition time of 600 s. Other conditions as in Fig. 1.

$$\mathrm{Zn(Bi)} \rightarrow \mathrm{Zn}^{2+} + 2e^-$$

This deduction can be further confirmed by the following test. After 0.5 μM Cd^{2+} and 0.5 μM Zn^{2+} were added to 1 μM MT in acetate solution of pH 4.0, the corresponding DPASV responses were recorded. Fig. 3B displays that the addition of the free Cd^{2+} and Zn^{2+} led to the much increase of peak currents at the same anodic peak potentials. Compared with those obtained at mercury electrodes [3], the DPASV responses of MT at BiFE were more concise without the peak originated from the complex of MT with electrode material. From the influences of solution pH on the DPASV responses of Cd^{2+} and Zn^{2+}, it can be seen that, in pH 2–7, the DPASV responses of Cd^{2+} were more suitable for the detection of MT. In the experiment below, we tested the DPASV responses of Cd^{2+} inherent to MT of different concentrations. Fig. 4 displays the calibration plot of DPASV peak currents for Cd^{2+} inherent to MT (I_p) versus MT concen-

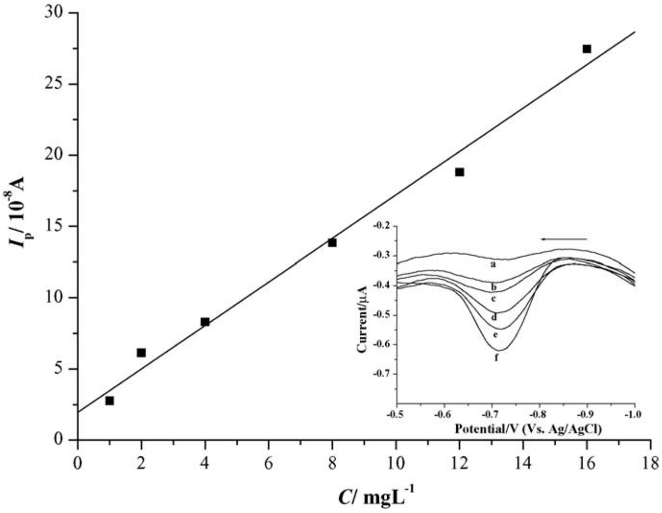

Fig. 4. Calibration plot of DPASV peak currents for Cd^{2+} inherent to MT (I_p) vs. MT concentrations (C). Inset: DPASV responses of MT at concentrations: (a) 1; (b) 2; (c) 4; (d) 8; (e) 12; (f) 16 mg/L.

trations (C). In the concentration range from 1 to 16 mg L^{-1}, a good linearity can be found with a correlation coefficient of 0.9936, a slope of 1.5260×10^{-8} AL/mg, and an intercept at 1.9507×10^{-8} A. The detection limit of MT was estimated as 3.86×10^{-8} mol/L (S/N = 3) under the experimental conditions of this paper. It can be concluded that BiFE is a good electrode for the detection of MT.

4. Conclusions

The preplated BiFE exhibited excellent performances for the DPASV detection of Cd^{2+} and Zn^{2+}inherent to MT. In pH 2–5.5, two well-defined anodic peaks corresponding to the Cd^{2+} and Zn^{2+} disassociated from MT were produced, which were more concise than those at mercury electrode etc. The calibration plot for MT detection showed a good linearity with a detection limit of 3.86×10^{-8} mol/L. It is suggested that, BiFE, a non-toxic excellent electrode, will be a good choice for the electrochemical study of metalloproteins.

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