

Full Paper

Voltammetric Studies of Cadmium- and Zinc-Containing Metallothioneins at Nafion-Coated Mercury Thin Film Electrodes

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Abstract

Voltammetric studies of rabbit liver metallothioneins (MTs, containing both Zn and Cd ions) and Zn₇-MT were carried out at Nafion-coated mercury film electrodes (NCMFs). The accumulation of MT molecules into the NCMFs enhances the voltammetric signals and the electrostatic interaction between the Nafion membrane and MT facilitates facile electron transfer reactions. Two well-defined redox waves, with reduction potential (E_{pc}) values at -0.740 and -1.173 V, respectively, were observed. The peak at $E_{pc} = -0.740$ V is attributable to the reduction of the Cd-MT complex, whereas that at $E_{pc} = -1.173$ V was assigned to the reduction of the Zn-MT complex. Zn₇-MT exhibits only one redox wave with $E_{pc} = -1.198$ V. The NCMFE was found to be more advantageous than thin mercury film electrode (MFE), because the pristine metal ions in MTs (e.g., Cd²⁺ and/or Zn²⁺) are not significantly replaced by Hg²⁺. The NCMFE is also complementary to Nafion-coated bismuth film electrode in that it has a greater hydrogen overpotential, which allows the reduction of the Zn-MT complex to be clearly observed. Moreover, intermetallic compound formation between Cd and Zn appears to be less serious at NCMFs. Consequently, the amounts of Cd and Zn deposited into the electrode upon the reduction reactions can be quantified more accurately.

Keywords: Metallothionein, Nafion-coated mercury film electrode, Voltammetry

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1. Introduction

Metallothioneins (MTs), long believed to play an important role in regulation of essential metals and detoxification of nonessential metals, are a type of proteins characterized by a large number of cysteine residues (up to 30% of the total amino acids), low molecular weight (ca. 7000 Da) and the absence of aromatic or histidine residues [1, 2]. Due to its remarkable chemical structure which confers a degree of specificity, stability, and dynamic behavior in terms of metal sequestration and transfer [3], MTs have captured the attention of biologists and chemists alike. Typically, MTs in their native states have a dumbbell-like structure comprising two clusters [1, 4, 5]. The metal ions in both clusters are tetrahedrally coordinated by both bridging and terminal thiolate ligands to form the metal-cysteine thiolates or metal-MT complexes. It is commonly known that apo-MT (MT without metals) can instantaneously bind to transition metal ions in the hierarchical binding sequence of $Hg^{2+} > Ag^+ > Cu^+ > Cd^{2+} > Zn^{2+}$ [6].

Recent studies have focused on the elucidation of the MT metal transfer process under various redox conditions or the quantification of the metal release associated with the MT redox reactions [7, 8]. Various electrochemical methods, such as cyclic voltammetry, square wave voltammetry, and differential pulse voltammetry, have been used to investigate the redox reactions of metal-MT complexes [9, 10].

Several groups have described the use of dropping mercury electrodes (DMEs) to examine the redox properties of MTs and their isoforms or peptide segments, as well as the metal/MT interaction [9–13]. Our group reported the redox behavior of rabbit liver MTs, which contain both Cd and Zn ions, adsorbed onto thin mercury films (MFEs) under different conditions. Two reversible redox peaks were observed at -0.63 and -0.91 V, respectively [7]. The peak at -0.63 V was attributed to the reduction of the Hg-MT complex formed between the adsorbed MT molecule and the mercury electrode, whereas that at -0.91 V was assigned to the reduction of cysteine–cysteine bonds (cystine analogues) in the MT adsorbate. Although the well-defined voltammetric peaks observed at MFEs led to an unambiguous determination of the redox potentials of mercury-containing MTs, the biological relevance is limited owing to the serious changes in the MT composition and structure resulted from the replacement of pristine metal ions by Hg. To alleviate these problems, we have developed Nafion-coated bismuth film electrodes (NCBiFEs) for voltammetric studies of MTs [14]. We found that the Nafion membrane pores match the size of the MT molecules. As a result, MT molecules can be accumulated into the membrane and the voltammetric signals were enhanced. More importantly, bismuth does not appear to bind strongly with MTs and the electrostatic interaction between the sulfonate groups of Nafion and the positively charged amino acid

residues of MTs (e.g., lysine) might have inhibited non-specific adsorption of MT molecules onto the bismuth surface. The main limitation of the NCBiFE is that the hydrogen overpotential was not sufficiently high, and the reduction of Zn-MT complex was overwhelmed by the high hydrogen reduction current. In the same report [14], we compared the voltammetric responses of MTs at both NCBiFEs and Nafion-coated mercury film electrodes (NCMFEs).

In this paper, we describe the use of the NCMFE for alleviating the extent of metal replacement. We show that the reduction reactions of both Cd-MT and Zn-MT complexes are diffusion-controlled at the NCMFE. The NCMFE was found to favor the accumulation of Cd and Zn generated from Cd-MT and Zn-MT complex reductions. Amounts of the metals reduced from the MT molecules were determined via the method of standard addition (with Cd^{2+} and Zn^{2+} standards). The quantification was facilitated by the lack of intermetallic compound formation at the NCMFE. Our work demonstrates that the NCMFE is complementary to the NCBiFE for the voltammetric studies of MTs and is more amenable to the studies of Zn-containing MT molecules and to the determination of redox-induced metal release from MTs.

2. Experimental

2.1. Chemicals and Solutions

Rabbit liver MTs (containing MT-I and MT-II) were obtained from Sigma (St. Louis, MO). The weight percents of Cd (ca. 3%) and Zn (ca. 1%) in the MT sample are specified by the manufacturer. Zn₇-MT was obtained from Hunan Lugu Biotech Co., Ltd (Changsha, China). MT solutions were prepared in a 10 mM phosphate buffer (pH 7.4) containing 1 mM sodium chloride. Mercury, zinc, and cadmium standard solutions were acquired from Aldrich Chemical Company, Inc. (Milwaukee, WI). Tris-(hydroxymethyl)aminomethane hydrochloride (Tris-HCl, Sigma) was used to prepare the Tris-HCl buffer solutions (0.08 M and pH 7.4). All solutions were prepared with deionized water treated with a Millipore water purification system (Simplicity Plus, Millipore Corp.). Nafion solutions (5% w/v solution in a mixture of alcohol and water, Aldrich Chemicals) were used as received.

2.2. Instruments

A three-electrode cell was used and the voltammetric experiments were conducted with a CHI 660B electrochemical workstation (CH Instruments, Austin, TX). The working electrode was a glassy carbon disk with a diameter of 3 mm. A platinum electrode and a Ag/AgCl electrode were used as the auxiliary and reference electrodes, respectively.

2.3. Procedures

The glassy carbon electrode for preparing the NCMFE was polished with alumina slurry down to 0.3 μm . The electrode was then sonicated in deionized water to rid any residual polishing materials off the surface. In a typical procedure, Nafion-coated mercury films were prepared by casting 3 μL of 1% Nafion solution onto the electrode surface and allowing the solution to dry. Mercury films were then deposited by holding the electrode potential at -0.4 V for 8 min in a N_2 -degassed 1% HNO_3 solution containing 0.5 mM Hg^{2+} . The incorporation of MT molecules into the pores present in NCMFEs was accomplished by casting 10 μL of a 38 μM MT solution on top of the electrode surface for a predetermined period of time (e.g., 1 h). To avoid water evaporation, the electrode was kept in a sealed Styrofoam box saturated with water vapor. After rinsing with deionized water, the MT-covered electrode was transferred to a Tris-HCl buffer for electrochemical determination. Prior to each measurement, the Tris-HCl buffer was thoroughly deaerated with N_2 for at least 10 min. The typical experimental parameters for differential pulse voltammetry (DPV) are 50 ms for the pulse width and 50 mV for the pulse amplitude.

3. Results and Discussion

Cyclic voltammograms (CVs) of rabbit liver MTs containing Cd (ca. 3%) and Zn (ca. 1%) acquired at three different types of electrodes are overlaid in Fig. 1. At the NCMFE, two well-defined redox waves were observed (solid line curve in Fig. 1). The cathodic and anodic peak potentials of wave 1 are -0.740 V (E_{pc1}) and -0.704 V (E_{pa1}), respectively, whereas those of wave 2 are -1.173 V (E_{pc2}) and -1.129 V (E_{pa2}), respectively. Several groups have reported the redox behaviors of MTs at DMEs and solid electrodes and generally assigned peaks between -0.6 and -0.9 V to the reduction of the Cd-MT complex or the cadmium-cysteine side chains [8, 9, 15–17] and the peaks between -1.0 and -1.3 V to the Zn-MT complex [9, 10]. We therefore assign wave 1 to the redox reaction of the Cd-MT complex and wave 2 to that of the Zn-MT complex. At a scan rate (v) of 0.1 V/s, the peak separations (ΔE_p) of these waves were determined to be 0.036 and 0.044 V, respectively. Such ΔE_p values suggest that the two redox waves are quasireversible [18]. These waves are either absent or difficult to discern at a Nafion-coated glassy carbon electrode (see the broad and ill-defined peak in the dashed line curve in Fig. 1) or a MFE (dotted line curve).

Previously we observed two reversible redox waves (E_{pc} values of -0.63 and -0.91 V) at MFEs that had been exposed to rabbit liver MT solutions for an extended period of time (e.g., 1 h) [7]. However, the peak positions are considerably different than the E_{pc1} and E_{pc2} values in the solid line curve of Figure 1. As reasoned before [7], an extensive exposure of MTs to the MFE results in a substantial replacement of the Cd and Zn metal ions by Hg. As a consequence, the composition of the adsorbed MT

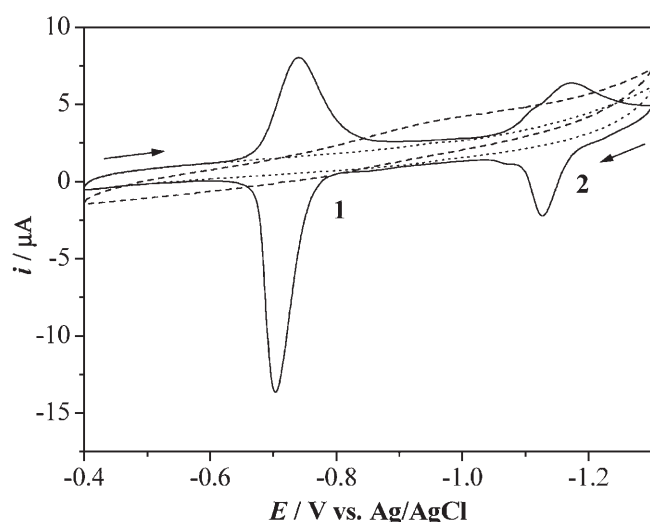


Fig. 1. Cyclic voltammograms of 38 μM MT adsorbed onto the various electrodes in 0.08 M Tris-HCl buffer solutions (pH 7.4) at a Nafion-coated mercury film electrode (solid line curve), a mercury film electrode (dotted line curve), and a Nafion-coated glassy carbon electrode (dashed line curve). Scan rate: 0.1 V/s.

molecules is significantly altered and the redox reaction of the resulting Hg-MT complex, instead of the reactions of Cd-MT and Zn-MT complexes was observed. Although the use of a glassy carbon electrode could mitigate the metal replacement, the electron transfer (ET) rate of MT is not facile (see the dashed line curve in Fig. 1). The use of NCMFE is therefore advantageous since efficient ET

reactions between the electrode surface and the Cd-MT and Zn-MT complexes are apparently facilitated. It thus appears that the Nafion membrane is capable of accumulating MT molecules and orientating the Cd-MT and Zn-MT complexes in such a configuration that facile ET can be obtained without significantly changing the MT composition.

We found that both the Nafion membrane and mercury film thicknesses affect the peak intensities. Our experiments indicate that casting 3 μL of the 1% Nafion solution onto the glassy carbon (GC) electrode to make a Nafion film and subsequently holding potential at -0.4 V in a 0.5 mM Hg^{2+} solution for 8 min to deposit mercury yielded the highest Cd-MT and Zn-MT peak currents (i_p). Furthermore, being consistent with our previously published voltammogram [14], the Hg-MT complex reduction is negligible. The stability of the NCMFE with MT molecules entrapped was also assessed. The reduction peaks remained essentially unchanged during consecutive potential scans, suggesting that the NCMFE that entraps MT molecules is stable. In fact, potential can be continuously cycled for as long as 10–15 min without appreciable signal degradation.

The plots of the i_{pc1} and i_{pc2} values against the square root of the scan rate ($v^{1/2}$) between 0.025 and 0.20 V are shown in Figure 2. These plots yielded i_{pc1} (μA) = $-1.725 + 22.08 v^{1/2}$ (V/s) ($r=0.9998$) and i_{pc2} (μA) = $-1.004 + 12.25 v^{1/2}$ (V/s) ($r=0.9987$), suggesting diffusion-controlled ET reactions. However, the plots of i_{pa1} and i_{pa2} versus v yielded i_{pa1} (μA) = $0.06144 + 10.35 v$ (V/s) ($r=0.9995$) and i_{pa2} (μA) = $-0.4397 + 31.80 v$ (V/s) ($r=0.9995$), indicating oxidations

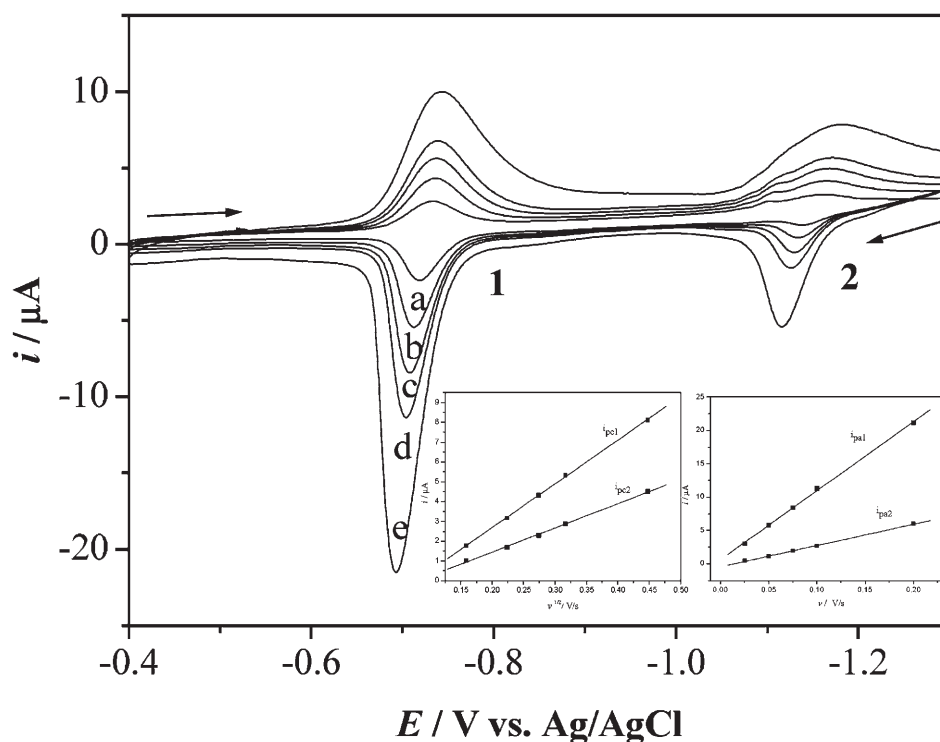


Fig. 2. Cyclic voltammograms of 38 μM MT at a NCMFE in a 0.08 M Tris-HCl buffer (pH 7.4) at the following scan rates: a) 0.025, b) 0.05, c) 0.075, d) 0.1, and e) 0.2 V/s. The insets show plots of i_{pc} and i_{pa} versus $v^{1/2}$ (left panel) and v (right panel), respectively.

of adsorbates at (from) the NCMFE [18]. As suggested in our previous report on the voltammetric studies of MTs at NCBiFEs [14], the comparable sizes between the MT molecules and the pores in the Nafion membrane [19] favor the accumulation or entrapment of the MT molecules in the membrane. Inside the membrane, there exist electrostatic attractions between the negatively charged sulfonates of Nafion and the positively charged MT amino acid residues (e.g., lysine groups). Such an electrostatic interaction appears to retard the adsorption of MT molecules onto the Hg surface or the Hg-MT complex formation. Consequently, the reductions of both the Cd-MT and Zn-MT complexes become diffusion-controlled. The shape of the voltammogram and the E_{pc} value for the Cd-MT complex reduction at the NCMFE are comparable to those at the NCBiFE [14]. We should point out that, due to the limited hydrogen overpotential of NCBiFE, the Zn-MT complex reduction peak could not be observed [14]. Thus, the use of NCMFEs expands the voltammetric studies of compositionally unperturbed MTs to the reduction of the Zn-MT complex.

A major difference between the NCMFEs and NCBiFEs is that the anodic peaks at the former are much more pronounced. Similar to other studies performed at DMEs or MFEs [8, 13, 17, 20], the anodic currents are attributable to the stripping of the Cd and Zn generated from the reductions of the Cd-MT and Zn-MT complexes and subsequently accumulated into the Hg film (in the forms of Cd-Hg and Zn-Hg amalgams). Such difference is conceivable if one considers that: 1) owing to the amalgam formation, accumulation of Cd and Zn into the Hg film is much more effective than into the Bi film and 2) Hg has a

much higher tendency of occupying the binding sites vacated by the Cd and Zn ions than Bi (recall that Hg has the highest binding affinity in the hierarchical order [6]). The sharp stripping peaks allowed us to quantify the amounts of metals released (vide infra), and provided an opportunity for voltammetric studies of redox-induced metal transfer process [21–23].

Figure 3 shows several MT linear sweep voltammograms collected at a NCMFE in the absence and presence of different amounts of Cd^{2+} standards spiked into the Tris-HCl buffer solution. As can be seen, i_{pa1} increased with the addition of Cd^{2+} . When Zn^{2+} was added into the solution, only i_{pa2} was enhanced (Inset). These observations further confirm our earlier assignments of the two anodic peaks, which are different than those at the NCBiFE [14]. At the NCBiFE, the addition of Zn^{2+} results in increases in both stripping peaks of Cd and Zn that are deposited after the reductions of the Cd-MT and Zn-MT complexes. Such increases are indicative of the Zn-Cd intermetallic compound formation. Previously we reported the Zn-Cd intermetallic compound formation upon MT redox reactions at the MFE [8]. Apparently, covering mercury film with a Nafion membrane suppresses the intermetallic compound formation. This is advantageous since the quantification of Cd and Zn released from the MTs could be achieved. Based on the standard addition method, quantities of Cd^{2+} and Zn^{2+} released during the Cd-MT and Zn-MT complex reductions were determined (Fig. 3). We found that, as soon as the potential scan was initiated at -1.3 V, $8.98 \mu M$ Cd ($n=4$, $RSD=2.0\%$) and $6.75 \mu M$ Zn ($n=4$, $RSD=1.2\%$) were released from the MT molecules confined within the Nafion membrane.

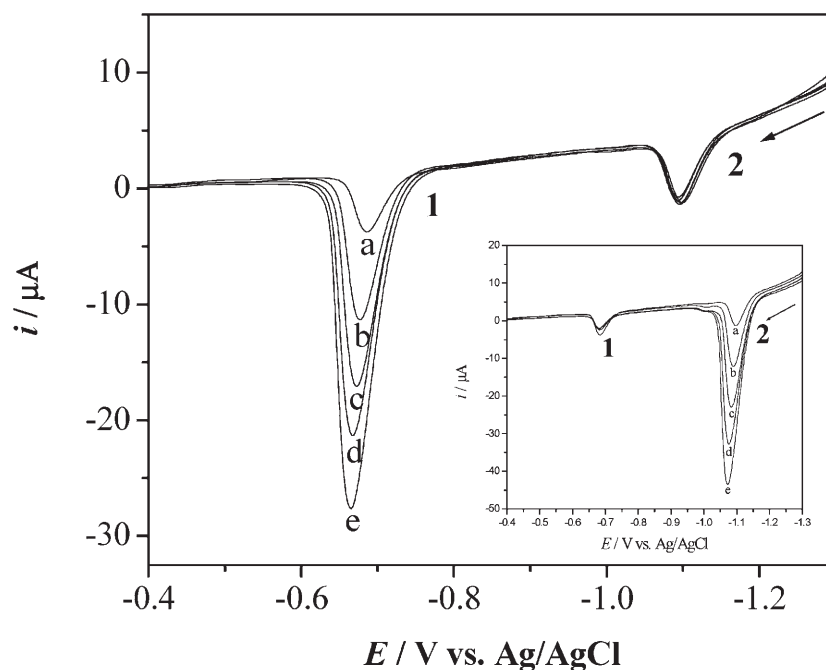


Fig. 3. Linear sweep voltammograms of $38 \mu M$ MT adsorbed onto a NCMFE for 1 h in a $0.08 M$ Tris-HCl buffer (pH 7.4) in the presence of different concentrations of Cd^{2+} : a) 0; b) $12 \mu M$; c) $18 \mu M$; d) $24 \mu M$; e) $36 \mu M$. The inset shows the presence of different concentrations of Zn^{2+} : a) 0; b) $6 \mu M$; c) $9 \mu M$; d) $12 \mu M$; e) $15 \mu M$.

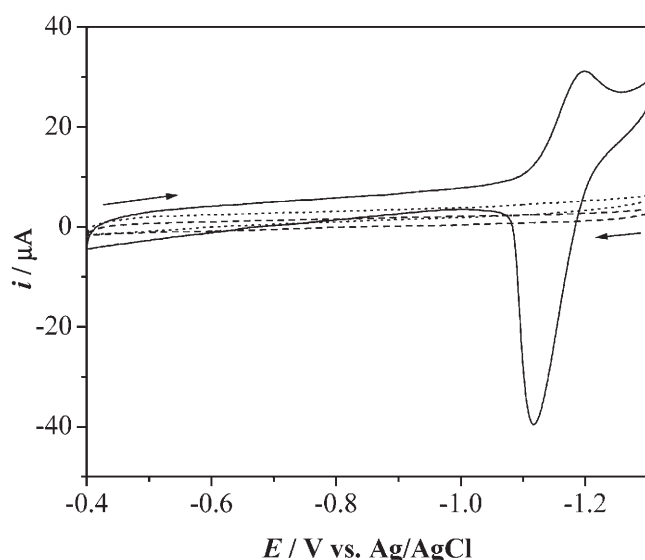


Fig. 4. Cyclic voltammograms of 38 μM $\text{Zn}_7\text{-MT}$ adsorbed onto the various electrodes in 0.08 M Tris-HCl buffer (pH 7.4) at a Nafion-coated mercury film electrode (solid line curve), a mercury film electrode (dotted line curve) and a Nafion-coated glassy carbon electrode (dashed line curve). Scan rate: 0.1 V/s.

Many isoforms of MTs contain Zn^{2+} [24]. For $\text{Zn}_7\text{-MT}$ entrapped in the NCMFE, only one voltammetric wave, with $E_{\text{pc}} = -1.198$ and $E_{\text{pa}} = -1.116$ V, was observed (solid line curve in Figure 4). Notice that these potentials are rather close to $E_{\text{pc}2}$ (-1.173 V) and $E_{\text{pa}2}$ (-1.129 V) of the rabbit liver MTs at the NCMFE (solid line curve of Fig. 1). Thus, it is clear that the reduction peak of wave 2 in Figure 1 is due to the Zn-MT complex. Notice that both i_{pa} and i_{pc} values are substantially greater than those of wave 2 in Figure 1. This is expected since the Zn content in $\text{Zn}_7\text{-MT}$ is greater than that in the rabbit liver MT. We should also point out that the $i_{\text{pa}}/i_{\text{pc}}$ ratio (1.9) deduced from Figure 4 is also greater than the $i_{\text{pa}2}/i_{\text{pc}2}$ ratio (1.1) for the Zn-MT complexes in rabbit liver MTs, suggesting that a greater amount of Zn was electrogenerated from the reduction of $\text{Zn}_7\text{-MT}$ and subsequently accumulated into the mercury film. Clearly, a NCMFE is highly desirable for voltammetric studies of Zn-containing MT isoforms owing to its high hydrogen overpotential. In Figure 4, we again contrasted the $\text{Zn}_7\text{-MT}$ voltammogram collected at a NCMFE to those acquired at a MFE (dotted line curve) and a Nafion-coated glassy carbon electrode (dashed line curve). Evidently, either an unfavorable orientation or insufficient adsorption of $\text{Zn}_7\text{-MT}$ impeded the ET reaction of $\text{Zn}_7\text{-MT}$ at the MFE and/or the Nafion-coated glassy carbon electrode, leading to featureless voltammograms.

4. Conclusions

In this work, NCMFEs have been utilized to explore the redox properties of two different Zn-containing MT isoforms. Due to the incorporation of MT into the Nafion

membrane, facile ET reactions between the MT molecules and the underlying mercury film can be achieved. Two well-defined redox waves were observed for rabbit liver MTs. The peak at -0.740 V was attributed to the reduction of the Cd-MT complex, whereas that at -1.173 V was assigned to the reduction of Zn-MT complex. For MT comprising only Zn^{2+} ($\text{Zn}_7\text{-MT}$), only the wave at the more negative potential was observed. Similar to the Nafion-coated bismuth film electrode (NCBiFE), nonspecific adsorption of MT onto the mercury film is alleviated by the electrostatic interactions between the Nafion membrane and the MT molecules. NCMFEs and NCBiFEs are complementary in that the former allow the reduction peaks of Zn-MT complexes to be observed while the latter do not cause replacement of the pristine metal ions (i.e., Cd^{2+} and Zn^{2+}). Moreover, since the Cd-Zn intermetallic compound formation is less severe at NCMFEs and the mercury film can effectively accumulate the reduced metal ions, amounts of Cd and Zn released from the MTs upon the Cd-MT and Zn-MT complex reductions can be quantified.

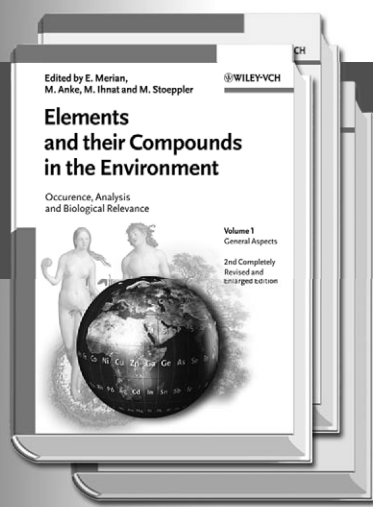
5. Acknowledgements

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6. References

- [1] J. Chan, Z. Huang, M. E. Merrifield, M. T. Salgado, *Coord. Chem. Rev.* **2002**, 233–234, 319.
- [2] M. Dabrio, A. R. Rodríguez, G. Bordin, M. J. Bebianno, M. D. Ley, I. Šestáková, M. Vasak, M. Nordberg, *J. Inorg. Biochem.* **2002**, 88, 123.
- [3] P. Coyle, J. C. Philcox, L. C. Carey, A. M. Rofo, *Cell. Mol. Life Sci.* **2002**, 59, 627.
- [4] A. H. Robbins, D. E. McRee, M. Williamson, S. A. Collett, N. H. Xuong, W. F. Furey, B. C. Wang, C. D. Stout, *J. Mol. Biol.* **1991**, 221, 1269.
- [5] N. Romero-Isart, M. Vasak, *J. Inorg. Biochem.* **2002**, 88, 388.
- [6] D. H. Hamer, *Ann. Rev. Biochem.* **1986**, 55, 913.
- [7] F. Song, A. L. Briseno, F. Zhou, *Langmuir* **2001**, 17, 4081.
- [8] A. J. Baca, Y. Garcia, A. L. Briseno, F. Zhou, *J. Electroanal. Chem.* **2001**, 513, 25.
- [9] A. R. Rodríguez, M. Esteban, *Cell. Mol. Biol.* **2000**, 46, 237.
- [10] I. Šestáková, P. Mader, *Cell. Mol. Biol.* **2000**, 46, 257.
- [11] M. Erk, B. Raspor, *Cell. Mol. Biol.* **2000**, 46, 269.
- [12] C. Harlyk, G. Bordin, O. Nieto, A. R. Rodríguez, *J. Electroanal. Chem.* **1998**, 446, 139.
- [13] C. Harlyk, O. Nieto, G. Bordin, A. R. Rodríguez, *J. Electroanal. Chem.* **1998**, 458, 199.
- [14] M. Xu, Y. Wu, J. Wang, F. Zhou, *Electroanalysis* **2006**, 18, 2088.
- [15] R. Olafson, *Bioelectrochem. Bioenerg.* **1988**, 19, 111.
- [16] C. Ruiz, A. R. Rodríguez, *Anal. Chim. Acta* **1997**, 350, 305.
- [17] M. Fedruco, I. Šestáková, *Bioelectrochem. Bioenerg.* **1996**, 40, 223.
- [18] A. J. Bard, L. R. Faulkner, *Electrochemical Methods. Fundamentals and Applications*, Wiley, New York **2001**.

- [19] G. Kefala, A. Economou, A. Voulgaropoulos, *Analyst* **2004**, 129, 1082.
- [20] A. L. Briseno, F. Song, A. J. Baca, F. Zhou, *J. Electroanal. Chem.* **2001**, 513, 16.
- [21] W. Maret, B. L. Vallee, *Proc. Natl. Acad. Sci. USA* **1998**, 95, 3478.
- [22] L. J. Jiang, W. Maret, B. L. Vallee, *Proc. Natl. Acad. Sci. USA* **1998**, 95, 3483.
- [23] M. Brouwer, P. Whaling, D. W. Engel, *Environ. Health Perspec.* **1986**, 65, 93.
- [24] Y. Kojima, J. H. R. Kägi, *Trends Biochem. Sci.* **1978**, 3, 90.



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