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PROTEIN-BASED ELECTROCHEMICAL BIOSENSOR FOR DETECTION OF SILVER(I) IONS

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Abstract—Silver(I) ions are extremely toxic to aquatic animals. Hence, monitoring of these ions in the environment is needed. The aim of the present study was to suggest a simple biosensor for silver(I) ions detection. The suggested biosensor is based on the modification of a hanging mercury drop electrode (HMDE) by the heavy metal binding protein metallothionein (MT) for silver(I) ions detection. Metallothionein accumulated for 120 s onto the HMDE surface. After rinsing the electrode, the biosensor (MT modified HMDE) was prepared prior to detection of silver(I) ions. The biosensor was immersed in a solution containing silver(I) ions. These ions were bound to the MT structure. Furthermore, the electrode was rinsed and transferred to a pure supporting electrolyte solution, in which no interference was present. Under these experimental conditions, other signals relating to heavy metals naturally occurring in MT were not detected. This phenomenon confirms the strong affinity of silver(I) ions for MT. The suggested biosensor responded well to higher silver(I) ion concentrations. The relative standard deviation for measurements of concentrations higher than 50 μM was approximately 2% ($n = 8$). In the case of concentrations lower than 10 μM, the relative standard deviation increased to 10% ($n = 8$). The detection limit (3 signal/noise) for silver(I) ions was estimated as 500 nM. Environ. Toxicol. Chem. 2010;29:492–496. © 2009 SETAC

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INTRODUCTION

Heavy metal ions occur naturally in the Earth's crust and therefore are present throughout the environment [1]. In aquatic ecosystems, heavy metal ions accumulate, and their distribution and bioavailability are higher compared with terrestrial ecosystems. Silver(I) ions are extremely toxic to aquatic animals [2–7]. The organisms viewed as most sensitive to silver(I) ions are small aquatic invertebrates, particularly in the embryonic and larval stages [8–12]. Toxicity of silver(I) ions occurs mainly in the aqueous phase and depends on the concentration of active, free Ag^+ ions. Toxicity of these ions relates to their high affinity for nucleic acids and proteins, which can result in damage to live sustaining biochemical pathways [13]. In 1991, the U.S. Environmental Protection Agency deleted the maximal contaminant level for silver in drinking water (an enforceable value) and replaced it with a secondary maximal contaminant level goal (nonenforceable) of 100 μg/L that is twice the previous level, an action that was driven by the recognized low toxicity associated with silver [14].

Monitoring heavy metal ions in situ is still difficult. Precise but expensive instruments requiring labor- and time-consuming sample preparation, such as spectrometric apparatus, are most commonly used for detecting heavy metals in environmental samples. Consequently, new and less demanding procedures for their rapid and sensitive detection are being developed [15–20]. Biosensors, instruments consisting of biologically active substances and physical transducers, have the advantages of specificity, low cost, ease of use, portability, and the ability to furnish continuous real-time signals [16]. Several recently published papers have described determination of heavy metals using electrochemical biosensors based on their interactions with DNA, enzymes, proteins, and whole cells [1,2,15–19,21–30]. The aim of the present study is to propose a simple biosensor for silver(I) ion detection.

MATERIALS AND METHODS

Chemicals and material

Rabbit liver metallothionein (molecular weight 7,143 g/mol) containing 5.9% Cd and 0.5% Zn, silver nitrate ($AgNO_3$), and all other reagents used were purchased from Sigma Aldrich. All chemicals met the purity specifications of the American Chemical Society (ACS) unless otherwise noted. Stock standard solutions were prepared with ACS water and stored in the dark at −20°C. Metallothionein standards were reduced by 1 mM tris(2-carboxyethyl)phosphine prior to use, because reduced

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metallothionein offers better repeatability and higher sensitivity of a determination compared with nonreduced metallothionein [1]. Working standard solutions were prepared daily by dilution of the stock solutions. All solutions were filtered through 0.45-μm nylon filter discs (MetaChem).

Electrochemical measurement

Differential pulse voltammetric measurements were performed with an Autolab Analyzer (EcoChemie) connected to VA-Stand 663 (Metrohm), using a standard cell with three electrodes. The three-electrode system consisted of a hanging mercury drop electrode (HMDE) with a drop area of 0.4 mm^2, an Ag/AgCl/3 mol/dm^3 KCl reference electrode, and a carbon counter electrode. The raw data were treated using the Savitzky and Golay filter (level 2) [31] and the moving average baseline correction (peak width 0.03), both implemented in General Purpose Electrochemical System (GPES) software 4.9 (EcoChemie). Differential pulse voltammetric parameters were as follows: initial potential of −1.2 V, end potential −0.3 V, modulation time 0.057 s, time interval 0.2 s, step potential 1.05 mV, and modulation amplitude 25 mV.

Descriptive statistics

Data were processed in Microsoft Excel®. Results are expressed as mean ± SD unless noted otherwise. The detection limits (3 signal/noise; S/N) were calculated according to Long and Winefordner [32], whereas N was expressed as standard deviation of noise determined in the signal domain.

RESULTS AND DISCUSSION

Metallothionein and its relation to heavy metal ions

Metallothionein is a low-molecular-mass protein playing a crucial role in transporting and maintaining homeostasis of heavy metal ions [1,23]. Because of its very specific structure, one molecule of this protein is able to bind up to seven divalent and 11 monovalent ions of heavy metals into cysteine clusters (Fig. 1). This unique property allows this protein to be a biological part of the biosensor for simple, selective, and sensitive heavy metal ions detection, mostly in areas with minimal laboratory facilities (in situ environmental monitoring, tropical and arctic territories).

MT-based biosensor for silver(I) ion detection

It has been shown previously that MT-based biosensors can be utilized for detection of various heavy metal ions and their compounds [1,26,33–35]. Metallothionein studied by differential pulse voltammetry gives several electrochemical signals: MT(Cd), MT(Zn), ZnT, and FreeT. The signal called FreeT

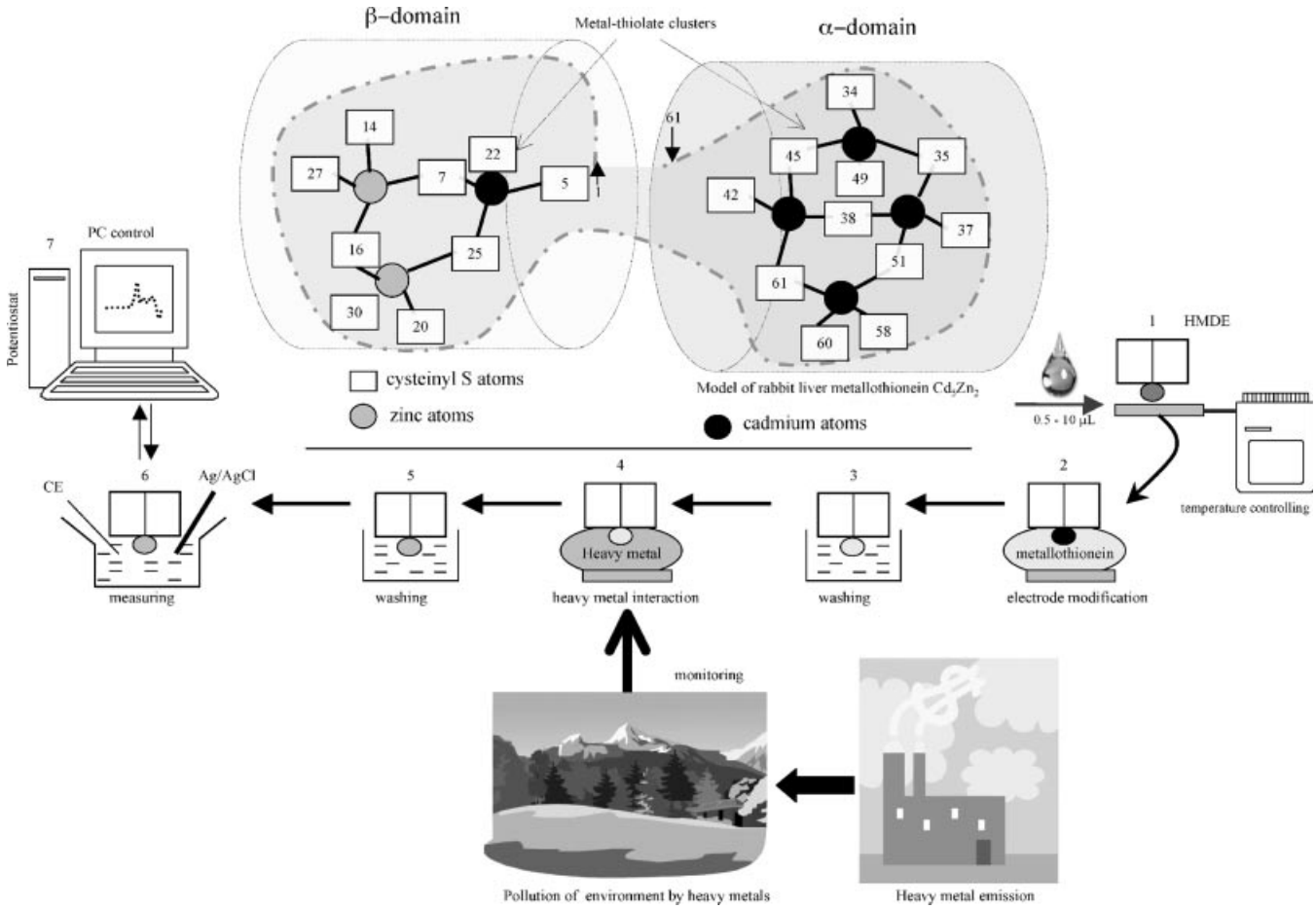

Fig. 1. Scheme of the adsorptive transfer stripping technique used for the suggested heavy metals biosensor; 1, renewing of the hanging mercury drop electrode (HMDE) surface; 2, adsorption of metallothionein in a drop solution onto the HMDE surface at open circuit (240 s); 3, rinsing of the electrode in supporting electrolyte; 4, interaction of heavy metal ions in a drop solution with the protein-modified HMDE surface at open circuit; 5, rinsing of the electrode in supporting electrolyte; 6, measuring; 7, data processing.

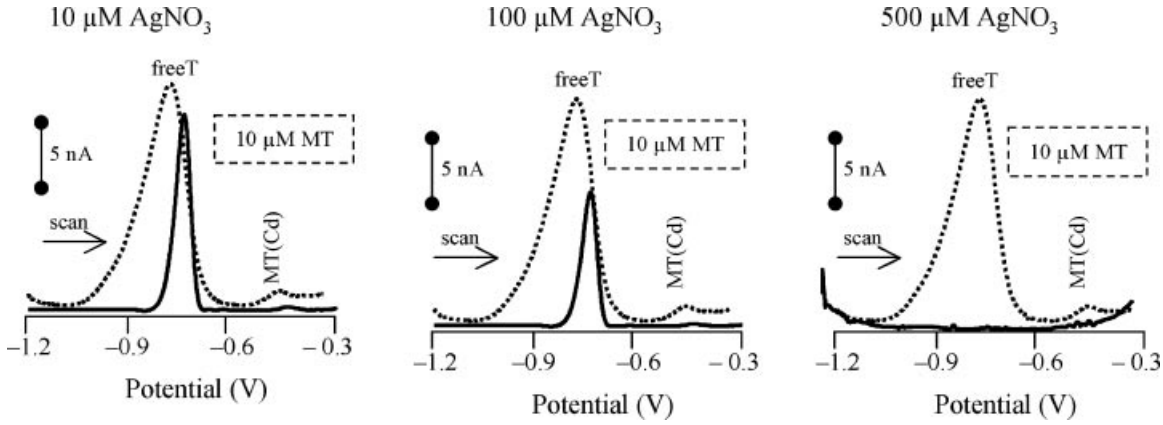

Fig. 2. Metallothionein (MT) studied by differential pulse voltammetry gives a few electrochemical signals: MT(Cd), MT(Zn), ZnT, and FreeT. The signal called FreeT probably corresponds to oxidation of the free thiols groups of metallothionein; the others relate to naturally contained zinc and cadmium ions in MT. To enhance sensitivity to silver(I) ions, we prepared apo-MT (protein without heavy metals) and used it in the following experiments [36]. After interaction, the present study showed a decrease in FreeT signal. Silver nitrate ($AgNO_3$) was used as a source of silver(I) ions.

probably corresponds to oxidation of the free thiol groups of metallothionein; the others relate to naturally contained zinc and cadmium ions. To enhance the sensitivity of silver(I) ions, we prepared apo-MT (protein without heavy metals) and used it in the following experiments [36]. The affinity of MT for silver is well known [37]. Therefore, the previously designed biosensor was tested for silver(I) ion detection. Metallothionein accumulated for 120 s onto the HMDE surface. After rinsing of the electrode, the biosensor (MT-modified HMDE) was prepared prior to detection of silver(I) ions. The biosensor was immersed in solution containing silver(I) ions. These ions were bound to the MT structure. Furthermore, the electrode was rinsed and transferred to a pure supporting electrolyte solution, in which no interference was present (Fig. 1). Metallothionein-modified HMDE provided a background signal (FreeT) in the presence of 0.5 M NaCl (pH 6.4; see Fig. 2). Moreover, changes after the interaction of MT-modified HMDE with various concentrations of silver(I) ions (10, 100, and 500 μM) are also shown. FreeT signal decreased with increasing silver(I) ion concentrations. At a silver(I) ion concentration of 500 μM, as a result of strong interaction with MT, the FreeT signal height was negligible (Fig. 2). Under these experimental conditions, other signals relating to heavy metals naturally occurring in MT were not detected. This phenomenon confirms the strong affinity of silver(I) ions for MT.

To study the interactions between MT and silver(I) ions in greater detail, dependences of FreeT peak height on interaction time of MT-modified HMDE with silver(I) ions were measured. Metallothionein (10 μM) accumulated on the HMDE surface for 120 s and further interacted with silver(I) ion concentrations of 10, 50, 100, and/or 500 μM (Fig. 3A). The shapes of the dependences obtained clearly demonstrated the rate of MT interaction with silver(I) ions. Points of break dividing the dependence into two parts were observed. At the lowest tested concentrations (10 and 50 μM), the point of break was not evident until a 600-s-long interaction. In the case of 100 or

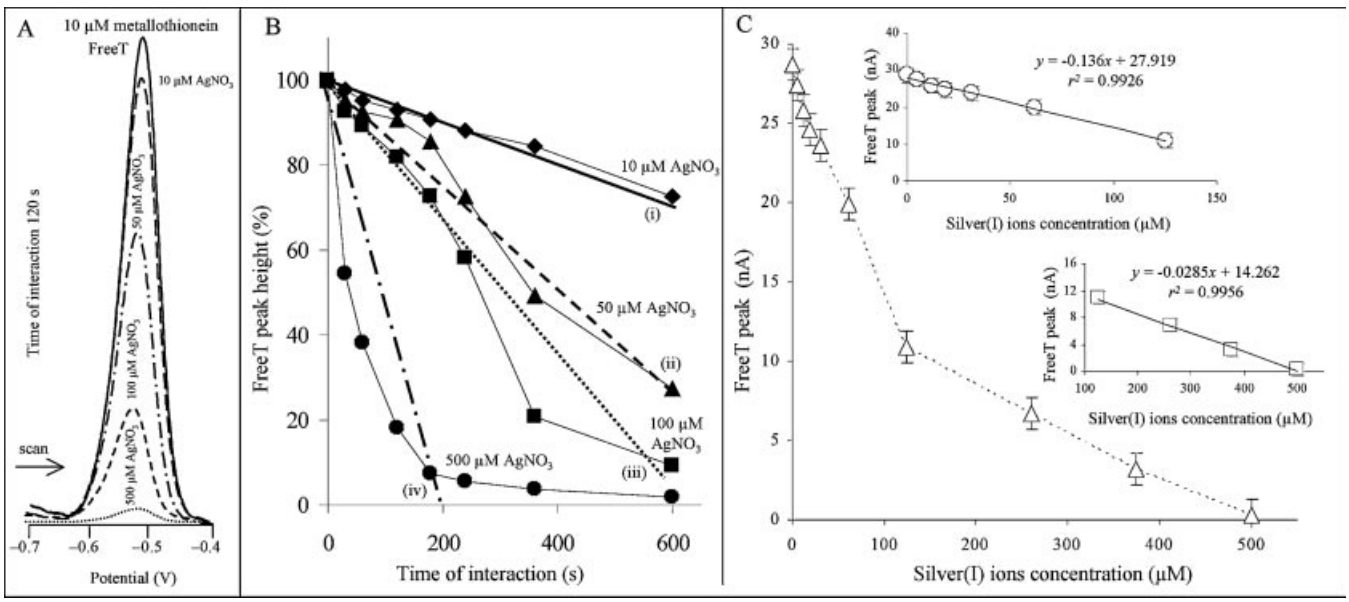

Fig. 3. (**A**) FreeT signals after interaction with silver(I) ions. (**B**) Influence of time of interaction on FreeT peak height. (**C**) Calibration curves for silver(I) ions within concentration range from 5 to 500 μM. Silver nitrate ($AgNO_3$) was used as a source of silver(I) ions.

500 μM silver(I) ions, the points of break were detected after 350- or 100-s-long interaction (Fig. 3B). To quantify the rate of interactions between MT and silver(I) ions, the dependences were plotted with straight lines, whereas the two last points were omitted from 500 μM dependence. Slopes of the straight lines are as follows: 10 μM −0.04 (i), 50 μM −0.126 (ii), 100 μM −0.164 (iii), and 500 μM −0.345 (iv; Fig. 3B). A slope of the tangent to a curve at a point shows the steepness of a curve. Based on the results of the present study, it can be concluded that the steepest straight line belongs to the curve describing 500 μM $AgNO_3$, which means the highest rate of interaction between MT and silver(I) ions.

Effect of silver concentration

In the experiments that followed, the dependence of FreeT peak height on silver(I) ion concentration within the range from 0 to 500 μM was determined. The dependence obtained is shown in Figure 3C. The shape of the response curve was exponential ($y = 31.2\ e^{-0.0078x}$). For analytical purposes, an attempt was made to divide the concentration interval into two parts. The first part [concentration of silver(I) ions = 100–500 μM] was strictly linear ($y = -0.0285x + 14.262$, $r^2 = 0.9956$). The second part [concentration of silver(I) ions = 0–125 μM] had the following parameters: $y = -0.136x + 27.919$, $r^2 = 0.9926$. The suggested biosensor responded well to higher silver(I) ion concentrations. Relative SD for measurements of concentrations higher than 50 μM was approximately 2% ($n = 8$). In the case of concentrations lower than 10 μM, relative SD increased to 10% ($n = 8$). The detection limit (3 S/N) for silver(I) ions was estimated as 500 nM. Given the fact that the maximum contaminant level in drinking water is approximately 1 μM, the proposed biosensor can be used for evaluation of pollution of the environment by these ions. One may suggest that spectrometric instruments with detection limits below nM level are more appropriate for detection of silver(I) ions [38–41]. Nevertheless, these instruments suffer from high cost and laborious sample preparation.

Moreover, the present study tested the biosensor on detection of silver(I) ions in waters naturally containing these ions, photographic emulsion ($n = 5$). This emulsion was purchased from Tesco. Based on standard additions, it was determined that this emulsion contained 1.6 ± 0.3 μM. This result is in good agreement with the manufacturer and with previously published data [42].

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