Full Paper

Palladium Biosensor

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Abstract
In this paper we proposed a palladium(II) biosensor. The biosensor is based on determining of interactions between palladium(II) and metallothionein modified hanging mercury drop electrode by means of differential pulse voltammetry. We studied influence of two supporting electrolytes (potassium or sodium chloride) on the signals of the biosensor. Based on the results obtained we found potassium chloride (0.05 M) as the most suitable supporting electrolyte to determine palladium(II). The detection limit of the biosensor for palladium ions was evaluated as 100 nM with *RSD* about 10%. Moreover, we utilized the biosensor for measurement of the target molecule in the presence of human blood serum and human urine.

Keywords: Palladium ions, Heavy metal, Electrochemistry, Electrochemical biosensor, Environmental analysis

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1. Introduction

The most important application of Pt and Pd together with Rh during the last 20 years has been connected with three-way catalytic converters for car. The emission of these metals into the environment is largely associated with the production and recycling of catalytic converters in the metal finishing industry as well as the operation of vehicle catalysts [1]. The literature surveys show that the concentration of platinum group metals in diverse environmental matrices has increased significantly over the last two decades [2–4]. Therefore, the amount of Pt and Pd released into the environment, their impact on human health, and routes for bioaccumulation and transformation in urban environment have to be monitored. Despite recent advances in instrumental techniques, the analysis of Pt and Pd in geological, industrial, biological and environmental samples is still a difficult task. Two main groups of analytical methods, spectroscopic [5–12] and voltammetric [9, 13–16], have been employing for detection of palladium(II) ions. Particularly, Locatelli showed that square wave adsorption stripping voltammetry represents excellent tool for ultra-trace detection of Pd(II) ions [14]. Nevertheless a sensor or biosensor for detection of palladium has not been proposed yet.

Based on our convincing results on the heavy metal biosensors [17–21], we focused on suggesting and utilizing of the biosensor to determine palladium. Particularly, the aim of this paper was to suggest palladium(II) biosensor based on interaction of this metal with metallothionein using adsorptive transfer stripping (AdTS) differential pulse voltammetry. Metallothionein (MT) belongs to group of intracellular and cysteine-rich proteins with molecular weight from 6 to 10 kDa, which are able to bind heavy metal ions via free −SH moieties [22]. MTs consist of two binding domains (α, β) that are assembled from cysteine clusters (Fig. 1A).

2. Experimental

2.1. Chemicals

Rabbit liver MT (MW 7143), containing 5.9% Cd and 0.5% Zn, was purchased from Sigma Aldrich (St. Louis, USA). Tris(2-carboxyethyl)phosphine (TCEP) is produced by Molecular Probes (Evgen, Oregon, USA). Sodium and potassium chloride, palladium chloride and other used chemicals were purchased from Sigma Aldrich. Stock standard solutions of MT (1 mM) were prepared by ACS

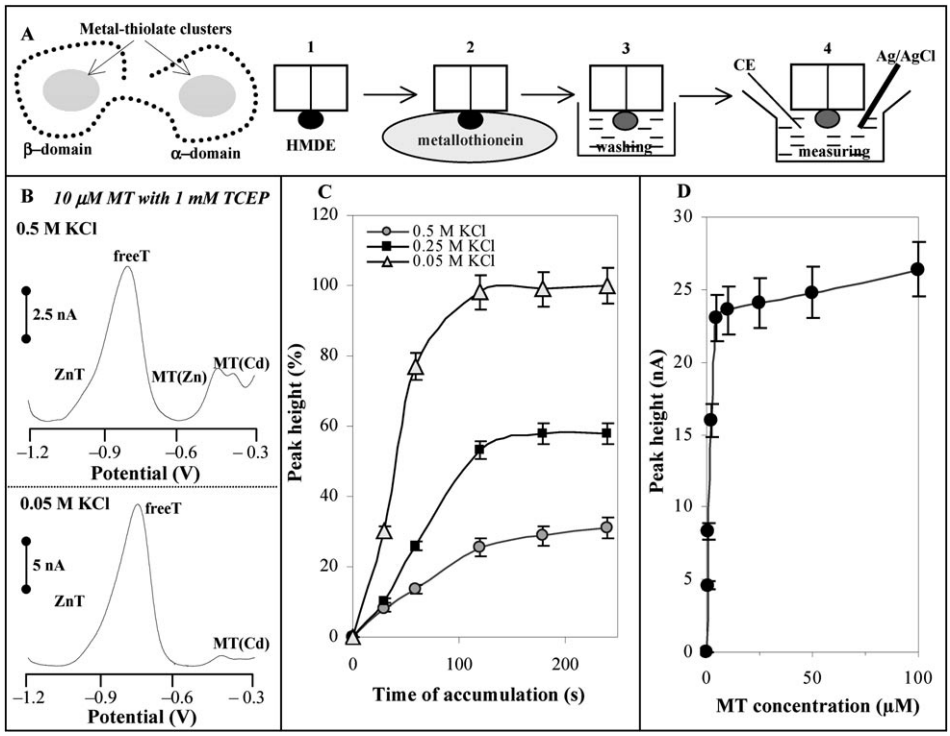

Fig. 1. A) Scheme of MT molecule and adsorptive transfer technique, Briefly, scheme of adsorptive transfer stripping technique used for suggestion of heavy metals biosensor; (1) renewing of the hanging mercury drop electrode (HMDE) surface; (2) adsorbing of MT in a drop solution onto the HMDE surface at open circuit (240 s); (3) washing electrode in potassium chloride (0.5 M, pH 6.4); (4) measurement of MT by DPV in potassium chloride. B) DP voltammograms of modified HMDE using adsorptive transfer technique. Experimental conditions: time of accumulation – 120 s, concentration of MT – 100 μM and supporting electrolyte – 0.5 M or 0.05 M KCl pH (6.4). C) Dependence of freeT signal height (MT – 100 μM) on different time of accumulation of MT onto surface of HMDE. D) Changes of electrochemical signal of freeT with increasing MT concentration. Experimental conditions: time of accumulation – 120 s. DPV parameters were as follows: the initial potential of – 1.2 V, the end potential – 0.3 V, the modulation time 0.057 s, the interval 0.2 s, the step potential of 1.05 mV, the modulation amplitude of 25 mV.

water (Sigma-Aldrich, USA) and stored in the dark at – 20 °C. Working standard solutions were prepared daily by dilution of the stock solutions. The pH value was measured using WTW inoLab Level 3 with terminal Level 3 (Weilheim, Germany), controlled by the personal computer program (MultiLab Pilot; Weilheim, Germany).

2.2. Electrochemical Measurements

Electrochemical measurements were performed with the AUTOLAB Analyzer (EcoChemie, Netherlands) connected to VA-Stand 663 (Metrohm, Switzerland), using a standard cell with three electrodes. The working electrode was a hanging mercury drop electrode (HMDE) with the drop area of 0.4 mm^2. The reference electrode was the Ag/AgCl/3M KCl electrode and the auxiliary electrode was the glassy carbon electrode. The analyzed samples were deoxygenated prior to measurements by purging with argon (99.999%), saturated with water for 120 s. All experiments were carried out at room temperature. For smoothing and baseline correction, the software GPES 4.4 supplied by EcoChemie was employed.

Adsorptive transfer stripping (AdTS) differential pulse voltammetry (DPV) – Palladium Biosensor. A detailed description of the metallothionein modification method has been previously published [18]. Briefly, scheme of adsorptive transfer stripping technique used for suggestion of heavy metals biosensor; (1) renewing of the hanging mercury drop electrode (HMDE) surface; (2) adsorbing of MT in a drop solution onto the HMDE surface at open circuit (240 s); (3) washing electrode in potassium chloride (0.5 M, pH 6.4); (4) interaction of palladium(II) ions in a drop solution with the protein modified HMDE surface at open circuit (this parameter was optimised, see Section 3.); (5) washing electrode in potassium chloride (0.5 M, pH 6.4); (6) measurement of MT by DPV in potassium chloride, pH 6.4. The MT standards were reduced by 1 mM tris(2-carboxyethyl)phosphine (TCEP) according [18, 23] prior to use, because reduced metallothionein offers better reproducibility and higher sensitivity of a determination in comparison with non-reduced ones due to presence of more free –SH groups [18]. The supporting electrolyte (sodium chloride: 0.5 M NaCl, pH 6.4) was purchased from Sigma Aldrich in ACS purity. DPV parameters were as follows: the initial potential of – 1.2 V, the end potential

– 0.3 V, the modulation time 0.057 s, the interval 0.2 s, the step potential 1.05 mV/s, the modulation amplitude 25 mV.

2.3. Real Samples

2.3.1. Human Urine

Human urine (obtained from healthy laboratory staff) was filtered through a Teflon disc filter (0.45 μm and 13 mm diameter, Alltech Associates, Deerfield, Il, USA) and 1000 times diluted with 0.05 M potassium chloride (pH 6.4) before measurements. We spiked 1000 times diluted solution of the human urine by Pd(II) at 2, 5, 10, 16, 25, 50 and 100 μM.

2.3.2. Human Blood Serum

Human blood serum samples were obtained from the Department of Clinical Biochemistry, University Hospital Ponavka in Brno, Czech Republic. Human blood serum was 1000 times diluted with 0.05 M potassium chloride (pH 6.4) before measurements. We spiked 1000 times diluted solution of the human urine by Pd(II) at 1.5, 3, 6, 12, 25, 50 and 100 μM.

3. Results and Discussion

3.1. Signals of MT Biosensor Affected by Supporting Electrolyte

The MT electrochemical biosensor has been utilized for various purposes previously [17, 18, 20], but an investigation of affecting of MT biosensor signals by changing of type and molar concentration of supporting electrolyte has not been done yet. Previously we have used sodium chloride as the supporting electrolyte, thus, we were interested in the issue how the exchange of the electrolyte for potassium chloride would influence the MT biosensor signals. Briefly, a hanging mercury drop electrode (HMDE) as working one was modified by MT (100 μM) at accumulation time of 120 s using adsorptive transfer stripping technique (Fig. 1A) and measured in the presence of potassium chloride (pH 6.4). The transfer technique was discovered Palecek and his colleagues by to prevent interferences, whereas they brilliantly improved the adsorptive stripping technique by so-called transfer step [24]. The main improving is based in electrode removing from a solution after accumulating of a target molecule on its surface, rinsing of the electrode and transferring to a pure supporting electrolyte, where no interferences are present.

The typical signals of commercially available $MT_{Cd,Zn}$ such as the signal corresponding to free –SH groups of MT called freeT (formerly called as CdT), the signal of relating to interaction between cadmium(II) ions and MT called Cd(MT) and the signal cadmium(II) ions bounded into MT called MTCd were observed (Fig. 1B). When we confirmed that MT gave the very similar signals in both potassium and sodium chloride, the investigation of affecting of MT biosensor signals by various molar concentrations of potassium chloride (0.05, 0.25 and 0.5 M KCl) followed. The various concentrations of the electrolyte changed markedly the height of signals observed (Fig. 1B). Moreover, the electrode was modified by MT at various times of accumulation in the presence of three concentrations of potassium electrode. The dependences obtained are shown in Fig. 1C. Based on the results obtained the highest current responses of MT biosensor were giving in the presence of 0.05 M KCl, whereas the responses were two and/or five times higher in comparison with those measured in 0.25 and/or 0.5 M KCl, respectively (Fig. 1B,C). In the latter potassium chloride (0.05 M) as supporting electrolyte was used. In addition, we studied the dependence of height of freeT signal on concentration of MT accumulated on the surface of HMDE (Fig. 1D). The height of freeT peak increased up to 10 μM concentration of MT sharply. At concentrations of MT above this value the peak height increased much more gradually. In the conclusion we choose the most suitable experimental conditions to suggest MT biosensor as follows: supporting electrolyte – 0.05 M KCl and MT concentration – 10 μM. The enhancing of the adsorptive stripping currents with the lowering the ionic strength of the test solution was observed also by Wang and Farias [25].

3.2. Interaction of MT Biosensor with Palladium(II) Ions

The MT biosensor was subsequently utilized to determine palladium. Voltammograms of MT biosensor without and with two concentrations of palladium(II) ions (100 and 500 μM) are shown in Figure 2A. The new signal called Pd(MT) well corresponded to binding of Pd(II) ions into MT. The new signal called Pd(MT) shown in the voltammograms in Fig. 2A can be associated to binding of Pd(II) with MT, because this signal appears only after interaction of the biosensor with palladium(II) ions. One may suggest that the signal could be also associated with reduction of palladium(II) ions itself. But if we accumulated (120 s) only free heavy metal ions (such as Cd(II) and/or Zn(II)) on the surface of an unmodified mercury working electrode, we did not observe any signal corresponding to heavy metal species [18]. Thus it can be concluded that the new signal called Pd(MT) associates with interaction of MT biosensor and Pd(II) ions. In addition Vasak and Kagi showed that the MT-heavy metal complexes are chemically and structurally uniform with binding of each bivalent metal to four thiolate ligands arranged in tetrahedral-like symmetry [26]. Thus, we may expect the same complex symmetry here.

When we confirmed that we were able to observe the interaction between MT biosensor and palladium(II) ions, an investigation of influence of freeT and Pd(MT) peaks height by various times of interaction at three Pd(II) ions concentrations (50, 100 and 500 μM) followed. The dependences are shown in Figure 2B,C. With increasing concentration of palladium(II) and time of interaction signal of

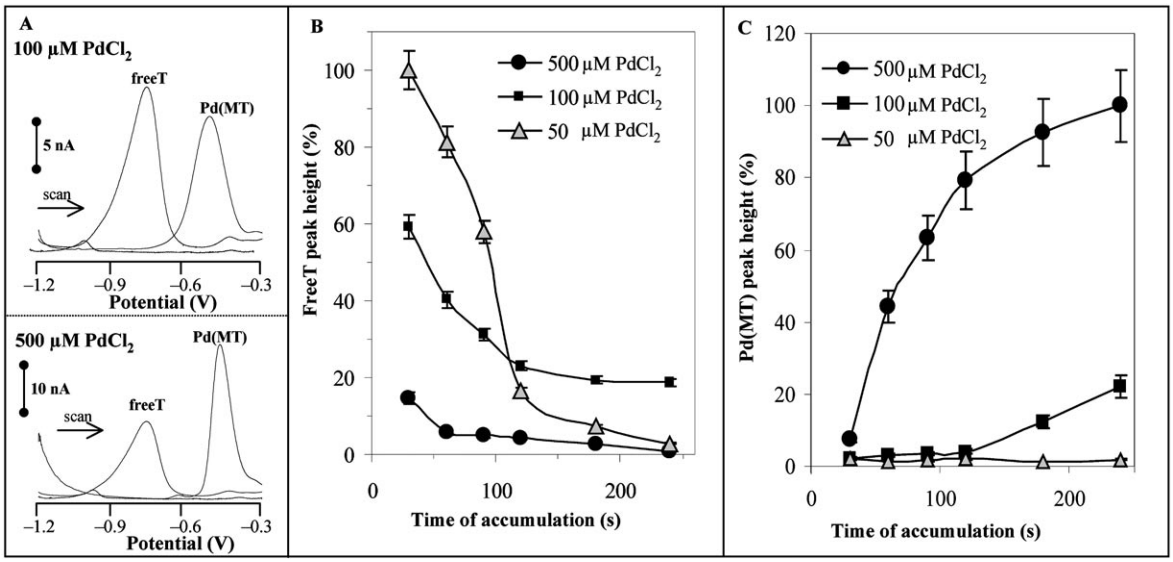

Fig. 2. A) DP voltammograms of MT biosensor (MT – 10 μM – accumulated onto surface of HMDE for 120 s; freeT) and MT biosensor after interaction (180 s) with 100 or 500 μM palladium ions ((Pd(MT)), bottom line corresponds to the supporting electrolyte – 0.05 M KCl. Affecting of signals of MT biosensor by various times of accumulation at three concentrations of palladium(II) ions (50, 100 and 500 μM): B) freeT signal and C) Pd(MT) signal. Other details see in Figure 1.

freeT decreased but Pd(MT) signal increased. Based on the results obtained it is clear that under the highest palladium(II) concentration the marked changes were observed (Fig. 2C).

3.3. Detection of Palladium(II) Ions by the Biosensor

The results obtained encouraged us to utilize MT biosensor to estimate linearity and detection limit of the biosensor. For these purposes MT was accumulated on the surface of HMDE for 120 s, whereas the interaction with the metal ions for 240 s followed (Fig. 3A). We found out that freeT signal decreased with increasing palladium(II) concentration. The decreasing dependence is not linear within the using concentration range, thus, we plotted the dependence by exponential curve ($y = 10.664e^{-0.018x}$). This exponential decrease could be associated with the depleting of free −SH groups able to bind with Pd(II) ions. One MT molecule is able to bind up to seven divalent ions, but due to adsorption

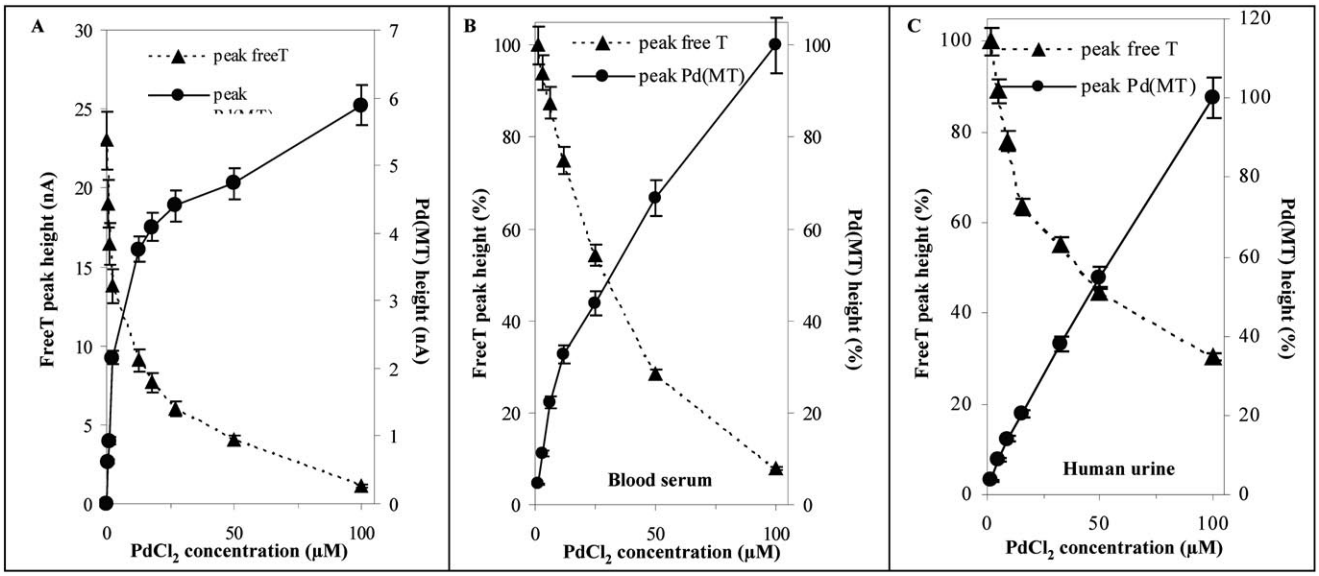

Fig. 3. Dependence of current responses of freeT and Pd(MT) signals on palladium(II) ions concentrations measured in the presence of A) the supporting electrolyte, B) human blood serum and C) human urine. Time of interaction of the biosensor with palladium(II) ions: 120 s. Other details see in Figure 2.

Table 1. Influence of biological matrix (human urine and human blood serum) on palladium biosensor signals.

Sample [a]	Biosensor signal [b]	Concentration range of Pd(II) (μM)	Equation	R^2
Supporting electrolyte	Pd(MT)	0 – 5	$y = 0.7358x + 0.1695$	0.9925
		10 – 100	$y = 0.0209x + 3.78$	0.9834
Human blood serum	Pd(MT)	1.5 – 6	$y = 1.410x - 0.4000$	0.9978
		12 –100	$y = 0.2746x + 9.050$	0.9938
Human urine	Pd(MT)	1.5 – 6	$y = 1.516x + 0.2782$	0.9885
		12 – 100	$y = 0.5785x + 8.081$	0.9939

[a] The supporting electrolyte was 0.05 M KCl. [b] The Pd(MT) signal was used for quantification of the palladium ions.

of MTs on the surface of HMDE one may suggest that the ability to bind the heavy metal ions will be declined. The change in steepness of the curve for freeT peak can be observed between 25 and 50 μM of Pd(II) ions. This phenomenon could be associated with ability of MT to bind ca. four Pd(II) ions to four thiolate ligands arranged in tetrahedral-like symmetry [26]. The following gradual decrease can relate to other more complex nonspecific interactions between MT and Pd(II) ions.

On the other hand Pd(MT) peak increased with increasing concentration of palladium(II) ions. For analytical purposes we attempted to split the dependence obtained into two parts: within the range from 0 to 5 μM and within the range from 10 to 100 μM. The equations and correlation coefficients are shown in Table 1. The detection limit of MT biosensor for palladium ions was 100 nM with *RSD* about 10%. The sensitivity of the biosensor depends not only on the advantages of AdTS but also on affinity of metallothionein to palladium(II). Based on our experiences it can be concluded that affinity of metallothionein to platinum(II) is the strongest followed by cadmium(II), zinc(II) and palladium(II).

3.4. Determination of Palladium(II) Ions by the Biosensor in the Presence of Human Urine and/or Human Blood Serum

We evaluated our palladium biosensor by means of detection of Pd(II) in the presence of the biological matrix (human urine and/or human blood serum) by spiking procedure. To be specific, MT (10 μM) was adsorbed (120 s) on the HMDE surface and then the modified electrode interacted with Pd(II) in the presence of human urine (1000 × diluted) or human blood serum (1000 × diluted) for the time of 120 s. Subsequently, the electrode was washed (0.5 KCl) and placed into an electrochemical cell containing supporting electrolyte (0.05 M KCl; pH 6.4). Human urine and/or human blood serum contained spikes of Pd(II) 1.5, 3, 6, 12, 25, 50 and 100 μM. It clearly follows from the results obtained that two peaks appeared – freeT and MT(Pd) (not shown). The results correspond well with the voltammograms measured in the presence of the supporting electrolytes.

With increase of palladium(II) ions concentration the height of freeT decreased and Pd(MT) increased (Figs. 3B,C). The equations characterizing increase in freeT peak height within two concentrations ranges are shown in Table 1. The detection limits Pd(II) evaluated as 3 *S*/*N* ratio were 0.8 or 0.9 μM measured in the presence of human urine.or human blood serum, respectively.

4. Conclusions

It follows from the previously published papers that a hanging mercury drop electrode modified by heavy metal binding mammalian protein called metallothionein can be used for determination of cadmium(II) and zinc(II) ions [18], cisplatin [17] and Pt(II)-DNA adducts [20] using differential pulse voltammetry. Here, we report on utilizing of this biosensor for detection of palladium(II). We optimized the supporting electrolyte and interaction times. We attempted to use the biosensor to measure palladium(II) in the presence of human body liquids.

Compared to voltammetric method reported by Locatelli [9], the detection limit of our proposed technique is approximately three times higher. Nevertheless the advantage of the proposed technique bases on sensor system itself, because the measurement systems could provide quick, simple and low-cost on-field determination after appropriate miniaturization.

5. Acknowledgements

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6. References

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