USING OF ELECTROCHEMICAL BIOSENSOR FOR STUDY OF PLATINUM-DNA INTERACTIONS

VYUŽITÍ ELEKTROCHEMICKÉHO BIOSENSORU PRO STUDIUM INTERAKCÍ PLATINY A DNA

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ABSTRACT

The biological activity of the first platinum based cytostatic drug – cisplatin (*cis*-diamminedichloroplatinum(II)) was discovered in 1965. Since then hundreds of platinum(II) and platinum(IV) complexes have been synthesized and evaluated as anticancer agents over past 40 years. As a consequence of the employment of platinum based cytostatic drugs in tumour diseases treatment, it became necessary not only to detect them in biological samples but also to determine and study the Pt-DNA adducts. The aim of this work was to suggest an electrochemical biosensor based on connection of metallothionein (MT) modified hanging mercury drop electrode (HMDE) with adsorptive transfer stripping differential pulse voltammetry, which can be used for studying of platinum-DNA interactions. The detection limit of cisplatin was about 2.5 pmole in 5 μl (0.5 μM). Moreover we tested the influence of human blood serum as a complex biological matrix on the way of determination of cisplatin and found out that we were able to determine tens of picomoles of cisplatin (5 μl drop). Finally, we used metallothionein modified HMDE to study interaction between cisplatin and DNA. Detection limit of Pt-DNA adduct was 312.5 ng/ml.

Keywords: Biosensor, Metallothionein, Cisplatin, Blood serum, DNA, Pt-DNA adducts.

ABSTRAKT

Biologická aktivita prvního na platině založeného cytostatika – cisplatiny (*cis*-diammindichloroplatina(II)) byla objevena v roce 1965. Od této doby byly a jsou stovky platnatých a platičitých komplexů syntetizovány a ověřovány jako protirakovinová léčiva. Jako důsledek využívání platinových cytostatik v léčbě nádorových onemocnění se stalo nezbytné nejen detekovat tyto látky v biologických vzorcích ale také stanovovat a studovat adukty platiny s DNA, která je cílovou molekulou těchto léčiv. Cílem této práce bylo navrhnout elektrochemický biosensor založený na kombinaci metalothioneinem modifikované visící rtuťové kapkové elektrody (HMDE) s adsorptivní přenosovou rozpouštěcí diferenční

pulsní voltametrii, které bylo možné využít pro studium platina-DNA interakcí. Detekční limit cisplatiny byl 2.5 pmolů v 5 μl (0.5 μM). Dále jsme testovali vliv krevního séra na průběh stanovení cisplatiny a zjistili jsme, že jsme schopni stanovit desítky pmolů cisplatiny. Metalothioneinem modifikovaná HMDE byla také použita pro studium interakcí mezi cisplatinou a DNA. Detekční limit Pt-DNA aduktu byl stanoven na 312.5 ng/ml.

Klíčová slova: Biosensor, Metalothionein, Cisplatina, Krevní sérum, DNA, Pt-DNA adukty.

INTRODUCTION

The pollution of the environment with toxic metals is a result of many human activities, such as mining and metallurgy, and the effects of these metals on the ecosystems are of large economic and public-health significance [1,2], because these substances are non-biodegradable and retained by the ecological system [3]. Besides "standard" toxic metals such as cadmium, lead and mercury, which have been monitoring for many years, following the introduction of automobile catalytic converters the platinum group metals (platinum and rhodium) gain on increasing interest in environmental research [4-7]. In addition platinum complexes play an important role in the chemotherapy of various tumour diseases [8-11]. The biological activity of the first platinum based cytostatic drug – cisplatin (*cis*-diamminedichloroplatinum(II)), which is still one of the most frequently used cytotoxic agent, was discovered in 1965 by Rosenborg during his studies on the effects of an electric current on bacterial growth [12]. Since then hundreds of platinum(II) and platinum(IV) complexes have been synthesized and evaluated as anticancer agents over past 40 years.

As a consequence of the employment of platinum based cytostatic drugs in tumour diseases treatment, it became necessary not only to detect them in biological samples but also to determine and study the Pt-DNA adducts. There are many techniques, which have been used for the determination of platinum based cytostatic drugs such as HPLC coupled to different kinds of detectors [13-15] and/or electrochemical methods [16-18]. On the other hand a few techniques have been employed for the detection of Pt-DNA adducts [19-23]. In addition the Pt-DNA adducts biosensor have not been suggested yet.

In the present work, we applied the metallothionein (MT) modified electrode (heavy metals biosensor) to determine commonly used platinum cytostatics – cisplatin and Pt-DNA adduct. Furthermore we tested the influence of complex biological matrix (human blood serum) on the cisplatin determination.

MATERIAL AND METHODS

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 chloride, cadmium nitrate, zinc nitrate and

other used chemicals were purchased from Sigma Aldrich. Stock standard solutions of MT with 10 μg/ml were prepared by ACS water (Sigma-Aldrich, USA) and stored in the dark at the temperature of -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). The pH-electrode (SenTix- H, pH 0–14/3M KCl) was regularly calibrated by set of WTW buffers (Weilheim, Germany).

Electrochemical measurements

Electrochemical measurements were performed with the AUTOLAB Analyser (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 graphite electrode. The supporting electrolyte was prepared by mixing buffer components. The analysed 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.

Suggestion of heavy metals biosensor

A detailed description of the metallothionein modification method has been previously published [5]. 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 sodium chloride (0.5 M, pH 6.4); (4) interaction of cisplatin in a drop solution with the protein modified HMDE surface at open circuit (this parameter was optimised, see Results and discussion section); (5) washing electrode in sodium chloride (0.5 M, pH 6.4); (6) measurement of MT by DPV in 0.5 M sodium chloride, pH 6.4. The samples of the MT were reduced before each measurement by 1 mM tris(2-carboxyethyl)phosphine (TCEP) according to [24]. The supporting electrolyte (sodium chloride: 0.5 M NaCl, pH 6.4) was purchased from Sigma Aldrich in ACS purity. DPV parameters were as follow: 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/s, the modulation amplitude of 25 mV.

Real samples

Preparation of 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 1,000 times

diluted with 0.5 M sodium chloride (pH 6.4) before measurements. Moreover, we added cisplatin (10, 20, 40, 80, 160, 350, 450, 530 and 650 μM) to 1,000 times diluted solution of human blood.

Preparation of cisplatin

The chemotherapeutic drug cisplatin was synthesized and provided by Pliva-Lachema (Brno, Czech Republic) [25]. Stock standard solutions of cisplatin at 10 μg/ml were prepared by sodium chloride solution (0.5 M, pH 6.4) and stored in the dark at the temperature of -20 °C. Working standard solutions were prepared daily by dilution of the stock solutions.

Preparation of DNA adduct with cisplatin

dsDNA obtained from chicken erythrocytes (100 μg/ml) was modified in the presence of 10 mM $NaClO_4$ by cisplatin (375 μM). The experiments were conducted for 24 hours at the temperature of 25°C in the dark of a thermostatic box (Model TER – 5/1, Chirana, Brno, Czech Republic).

Purification of DNA adduct

The obtained DNA adduct was purified for the period of 24 hours by ultrafiltration (Microcon YM-30, Millipore). The DNA adduct was laid on Microcon YM-30 membrane and centrifuged (Eppendorf, 14 000 g) for 10 min at 20 °C. Each of the low-molecular compounds, such as free cisplatin and fragments of DNA present in the solution came through the nitrocellulose membrane. Consequently, the sample reservoir was turned round and centrifuged (14 000 g, 10 min, 20 °C). Afterwards, the modified DNA was obtained.

Statistical analysis

STATGRAPHICS® (Statistical Graphics Corp®, USA) was used for statistical analyses. Results are expressed as mean ± S.D. unless noted otherwise. Value of $p < 0.05$ was considered significant.

RESULTS AND DISCUSSION

Recently we have published results describing heavy metals biosensor (MT modified surface of HMDE) that we have used for the determination of cadmium and zinc [5]. Here, we were interested in the issue of how the differential pulse voltammetric record of MT modified electrode surface looks like in the presence of cisplatin.

Primarily we accumulated 10 μM reduced metallothionein, which naturally contains Cd(II) and Zn(II) in its clusters, on the surface of the HMDE for 240 s and analysed the

adsorbed MT by differential pulse voltammetry (Fig. 1 – dashed line). The highest observed signal called CdT (–0.65 V) corresponds to adduct of the MT with mercury on the surface of the HMDE (HS-peptide + Hg = HgS-peptide) [5,26]. In addition the MT(Cd) (–0.42 V) and MT(Zn) (–0.49 V) signals relates to reduction of bounded metal [27] were observed. Signals ZnT` (–0.87 V) and ZnT (–0.99 V) probably results from electrochemical reactions proceeding between MT, heavy metals and mercury electrode. Other details will be published elsewhere. Moreover we applied the MT modified HMDE to determination of cisplatin (interaction time was 420 s). Resulted voltammogram is shown in Fig. 1 (continuous line). We observed three signals: CdT at the potential of –0.66 V, ZnT` at –0.87 V and peak at –1.11 V called PtMT that could correspond to reduction of platinum bounded to metallothionein (Fig. 1). On the other hand MT(Cd), MT(Zn) and ZnT signals disappeared which probably relates with the replacing of cadmium and/or zinc by platinum in the structure of MT adsorbed on the HMDE surface.

For analytical purposes we studied the dependence of the PtMT peak and/or CdT signal heights on cisplatin concentration. The dependences were linear in the studied concentration range of 25 μM – 375 μM of cisplatin (Tab. 1). The detection limit (3 S/N) of cisplatin ($[Pt^{II}(NH_3)_2Cl_2]^0$) calculated from the decrease of CdT peak was about 2.5 pmole in 5 μl (0.5 μM) at the interaction time of 400 s.

Tab. 1 Influence of biological matrix (human blood serum) on determination of cisplatin by MT modified electrode surface

Sample	Detected compound	Concentration of the cisplatin [μM]	MT signals[c]	Equation	R^2
Supporting electrolyte[a]	Cisplatin	25 – 375	PtMT	y = 0.0154x + 0.0833	0.9939
			CdT[b]	y = -0.0683x + 32.848	0.9993
Human blood serum	Cisplatin	10 – 160	PtMT	y = 0.0098x - 0.0963	0.9953
		350 – 650	PtMT	y = 0.1046x - 34.424	0.9960
		10 – 650	CdT[b]	y = -0.0067x + 25.843	0.9906

[a] … Supporting electrolyte was 0.5 M NaCl.
[b] … The CdT signal was used for quantification of the detected compound.
[c] … For detailed description of the MT signals see Results and Discussion section and/or [5,27-31].

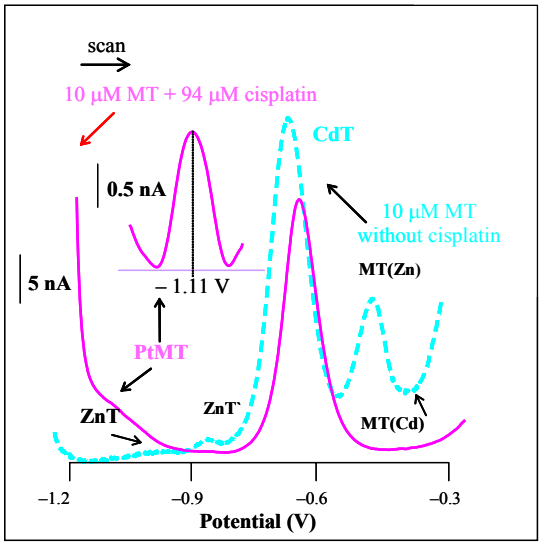

Fig. 1 Cisplatin – $[Pt^{II}(NH_3)_2Cl_2]^0$; anticancer drug – detection in 0.5 M NaCl. Typical DPV voltammograms of 10 μM MT without addition of cisplatin and 10 μM MT + 94 μM of cisplatin.

Determination of cisplatin in the presence of the human blood serum

For the study of the anti-cancer drugs effectiveness it is necessary to detect their therapeutic level. That is why we were interested in using the MT modified HMDE for determination of cisplatin in the presence of human blood serum. Hence, we added the different cisplatin concentrations (10, 20, 40, 80, 160, 350, 450, 530 and 650 μM) to the human blood serum samples. CdT signal specifically decreased with increasing cisplatin concentration (Tab. 1). Moreover, PtMT signal slowly increased up to cisplatin concentration 350 μM and then increased markedly. The detection limit (3 S/N) of cisplatin calculated from the decrease of CdT peak was about 2.5 μM (R.S.D. was 7.8 %, n = 5) at the interaction time of 400 s. The suggested approach shows the possible way for simple, sensitive and rapid detection of the anti-cancer drug in the human body fluids at the picomole level (10 pmole in 5 μl drop).

Finally, we used HMDE modified by MT to detect and quantify the Pt-DNA adducts. On the base of the results obtained, we selected 5 min as the most effective time of interaction with the view of rapid determination and relative high response. Detection limit of Pt-DNA adduct calculated from decreases of CdT signal was 312.5 ng/ml.

CONCLUSION

Electrochemical biosensors have superior properties over the other existing measurement systems because they can provide quick, simple and low-cost on-field determination of many biologically active species and a number of dangerous pollutants. Here we suggested a new biosensor to determine of platinum based cytostatic – cisplatin and to quantify Pt-DNA adducts.

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