

A sensor for investigating the interaction between biologically important heavy metals and glutathione

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ABSTRACT: Glutathione (GSH) is a water-soluble tripeptide playing a crucial role in the number of cellular reactions including detoxification of heavy metals. Glutathione can be found both in an oxidized (GSSG) and reduced (GSH) state. The aim of this paper was to use electrochemical methods to determine glutathiones and to investigate interactions of GSH and cadmium, zinc, copper and nickel ions or feed additive for animal nutrition. It clearly follows from the results that GSH and GSSG can be distinguished by cyclic voltammetry whereas the level of their current response is proportional to their concentration. Moreover, we studied these interactions by cyclic voltammetry. Marked changes in the GSH signal were observed and discussed. The highest decrease in a GSH reductive signal is caused by cadmium ions followed by zinc, nickel and copper ones. We also observed that the reductive GSH signal gradually decreased (about 9%) with the increasing concentration of feed additive used in our experiments. In conclusion, cyclic voltammetry offers a tool for studying interactions of thiols with different substances such as heavy metals and/or feed additives.

Keywords: reduced glutathione (GSH); oxidized glutathione (GSSG); thiols; cadmium; zinc; copper; nickel; additive; cyclic voltammetry; electrochemical method

Glutathione (GSH) is a water-soluble tripeptide that is composed of the amino acids γ -glutamine, cysteine, and glycine (Meister and Anderson, 1983) (see in Figure 1A). GSH belongs to the most abundant intracellular small molecules, reaching

up to 10^{-3} molar concentrations in some tissues (Meister and Anderson, 1983; Meister, 1988; Zehnálek et al., 2004b). The importance of GSH for living organisms of different types is evident from its abundance in plants, mammals, fungi and some

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prokaryotic organisms. Thanks to its thiol group GSH is a potent reducing agent, which is involved in the detoxification of various electrophilic compounds and peroxides as a cofactor of glutathione-S-transferases (GST) and glutathione peroxidases (GPx). In addition, GSH plays a crucial role in other cellular reactions including the glyoxalase system, reduction of ribonucleotides to deoxyribonucleotides, regulation of protein and gene expression via thiol:disulphide exchange reactions and many others (Meister, 1988; Kizek et al., 2004; Zehnálek et al., 2004a,b). This tripeptide can be found both in an oxidized (GSSG) and reduced (GSH) state. The maintenance of an optimal GSH:GSSG ratio in the cell is crucial to its survival, hence the tight regulation of the system is needed (Droge, 2002; Klejdus et al., 2004). A deficiency of GSH exposes the cell at risk of oxidative damage (Droge, 2002). It is not surprising that the imbalance of GSH can be observed in a wide range of pathologies including cancer, neurodegenerative disorders, cystic fibrosis (CF), HIV and aging. The determination of GSH and other sulphur-containing peptides and proteins like phytochelatins, metallothioneins (MT), MT-like proteins or metallochaperones is particularly urgent because of the high importance of these compounds in homeostasis metabolism and in the detoxification of both essential (Zn, Mn, Cu) and highly toxic heavy metals (Cd, As, Pb, Cr, etc.) (Vacek et al., 2004b). The heavy metal ions come into the environment from different sources (natural and anthropogenic ones) and consequently enter into the plant and animal tissues (Rous and Jelínek, 2000; Hejtmánková et al., 2002; Spurný et al., 2002; Svobodová et al., 2002, 2004; Piskorová et al., 2003; Sikirić et al., 2003; Bobček et al., 2004; Antunovic et al., 2005; Pintar et al., 2005; Skřivan et al., 2006; Váradyová et al., 2006). The heavy metals are distributed along the food chain and accumulated in predators including a man (Zehnálek et al., 2004a). After the consumption of heavy metals by an organism, they are covalently bound into biomolecules through the sulphhydryl (SH) groups of cysteine residues (Petrlová et al., 2006). Capillary electrophoresis (CE) or high-performance liquid chromatography (HPLC) are the most commonly used techniques for GSH separation. For the identification and determination of GSH and other related peptides and proteins electrochemical methods based on the catalytic evolution of hydrogen on mercury electrodes (peak H, Brdicka procedure) have been

intensively used (Heyrovský, 2000, 2004, 2006; Kizek et al., 2001; Mader et al., 2001; Brázdová et al. 2002; Trnková et al., 2002; Strouhal et al., 2003; Průša et al., 2004a,b, 2005, 2006a,b; Kukačka et al., 2006). Electrochemical techniques appeared to be very suitable for the design of sensors and biosensors (Billová et al., 2003; Kizek et al., 2003; Vacek et al., 2004a; Adam et al., 2005a,b,c; Potěšil et al., 2005, 2006).

The aim of this paper was to use electrochemical methods for the determination of reduced and oxidized glutathione. Particularly, the proposed methodology was applied to study the interactions of glutathione and biologically important heavy metal ions (cadmium, zinc, copper and nickel) both in standard and real samples. A feed additive for animal nutrition was used as a real sample.

MATERIAL AND METHODS

Chemicals

Reduced glutathione (GSH) and oxidised glutathione (GSSG) were purchased from Sigma Aldrich (St. Louis, USA). Other chemicals used including heavy metals were purchased from Sigma Aldrich unless noted otherwise. Stock standard solutions of GSH and GSSG (100 µg/ml) were prepared with 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 a of WTW buffers (Weilheim, Germany).

Electrochemical measurements

Electrochemical measurements were performed with an Autolab Analyser (EcoChemie, The 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 an Ag/AgCl/3M KCl electrode and the auxiliary one was a graphite electrode. All experiments were car-

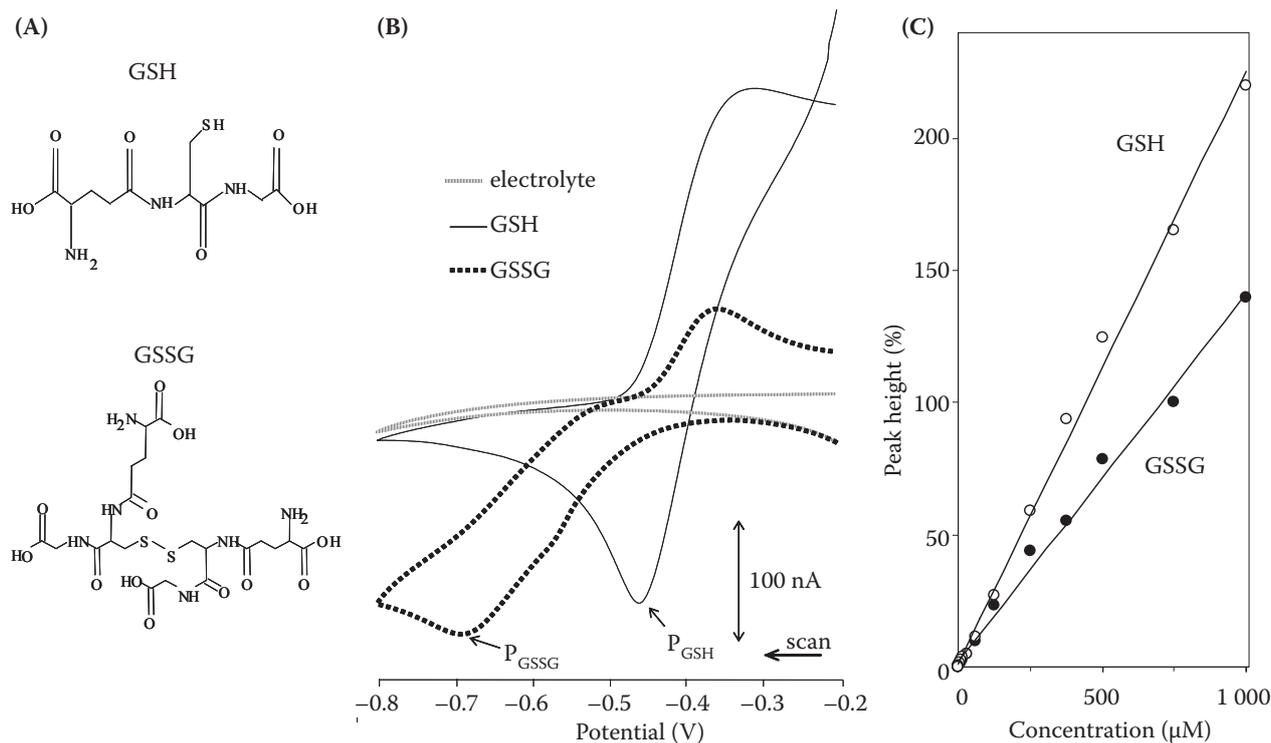


Figure 1. Electrochemical behaviour of reduced (GSH) and oxidized glutathione (GSSG)

(A) Chemical formulas of GSH and GSSG

(B) Typical cyclic voltammograms of supporting electrolyte, GSH and GSSG. Thiol concentration 100 μM and scan rate (25 mV/s)

(C) Dependence of the height of thiol signals on their concentration (0–20 μM). Supporting electrolyte (0.05M Na₂B₄O₇, pH 9.2). CV parameters were as follows: step potential 5 mV, starting potential -0.2 V, vertex potential -0.8 V, deoxygenation with argon for 200 s, scan rate 25 mV/s

ried out at room temperature. For smoothing and baseline correction, the software GPES 4.4 supplied by EcoChemie was employed. The GSH and GSSG were measured using cyclic voltammetry on the surface of HMDE. The supporting electrolyte (0.05M sodium tetraborate, pH 9.2) was purchased from Sigma Aldrich in ACS purity. CV parameters were as follows: initial potential -0.2 V, end potential -0.8 V and step potential 5 mV. The samples of GSH were reduced before each measurement by 1mM tris(2-carboxyethyl)phosphine addition according to Kizek et al. (2004) and Kukačka et al. (2006).

Feed additive

An additive to the feed of farm animals M0 was purchased from Mikrop Cebin (Czech Republic). Stock standard solutions of the additive (1 mg/ml) were prepared with ACS water (Sigma-Aldrich, USA) and stored in the dark at the temperature of -20°C.

RESULTS AND DISCUSSION

Cyclic voltammograms of thiols

Recently, we have proposed a simple electroanalytical method for reduced and oxidized glutathione determination (Kizek et al., 2004; Kukačka et al., 2006; Průša et al., 2006a). Under these experimental conditions the typical voltammograms of oxidized and reduced glutathione were obtained (Figure 1B). GSH gave a signal designated as P_{GSH} at a potential of -0.44 V, GSSG a signal designated as P_{GSSG} at -0.69 V. As for GSH, both reductive and oxidative signals are evident. The reductive signal corresponds to a reduction of the GS-Hg complex occurring on the surface of HMDE (Kizek et al., 2004; Vacek et al., 2004b). Particularly, we assume that thiols are firstly adsorbed on the working electrode surface and then a complex of thiol with mercury (GS-Hg) is formed. The electrode reaction of the thiol-mercury compound follows (Jin et al., 2000). The results clearly document that GSH and

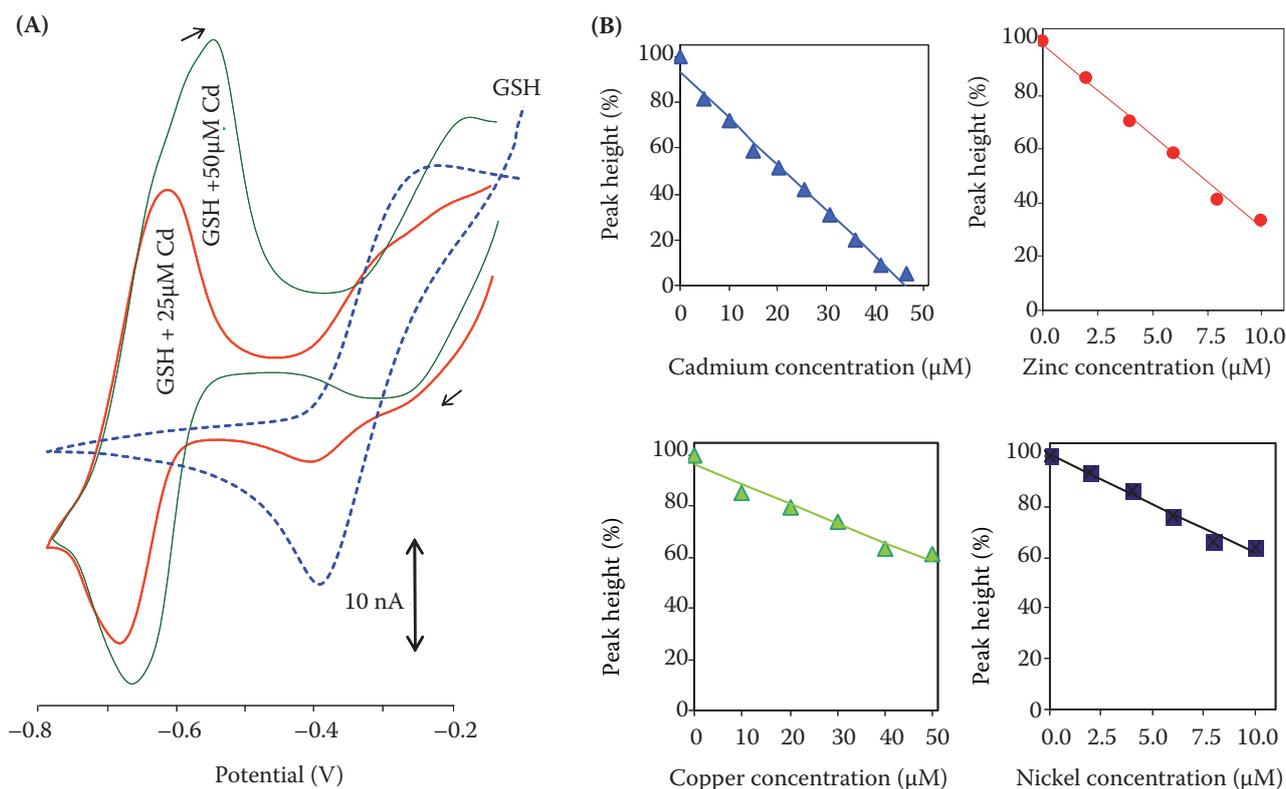


Figure 2. Cyclic voltammograms of GSH (dotted line) and GSH in the presence of 25 μM (thick solid line) and 50 μM (thin solid line) of cadmium ions (A) and (B) Dependences of GSH reductive signal on cadmium, zinc, copper and nickel concentrations

The peak height 100% represents 30 nA; for the other experimental conditions see Figure 1

GSSG can be distinguished by this method (Figure 1B). Moreover, the influence of a concentration (0–1.000 μM) on thiol signals was studied. The dependences were obtained at a scan rate of 25 mV/s by dilution of stock solutions of thiols and they were strictly linear (GSH, $y = 0.2246x + 0.962$, $R^2 = 0.9959$; GSSG, $y = 0.139x + 1.8416$, $R^2 = 0.9934$) with R.S.D. 5.8% (Figure 1C).

Investigation of glutathione interactions with heavy metals

It is common knowledge that glutathione can interact with heavy metal ions (Adam et al., 2005a,b,c). We studied these interactions by cyclic voltammetry. If we analysed GSH (100 μM) by CV, we obtained a typical voltammogram (Figure 1B). As we investigated GSH interactions with heavy metals, we added cadmium ions to the GSH solution and observed the changes. We found out that a reductive signal of GSH decreased and a signal cor-

responding to the glutathione-cadmium complex increased (–0.65 V) with the increasing concentration of heavy metal (Figure 2A). Moreover, we observed a strong oxidation signal at the potential of –0.6 V (Figure 2A). If we added cadmium (50 μM) to GSH (100 μM), we did not observe any reductive signal of the thiol. The results show that glutathione probably interacts with cadmium ions according to the following equation: $2\text{G-SH} + \text{Cd}^{2+} \rightarrow \text{G-S-Cd-S-G}$. The hypothesis confirms that GSSG gives an oxidation signal at the potential of –0.69 V in comparison with the GS-Cd-SG complex, which gives a signal at –0.65 V. This shift could be caused by cadmium ions bound to GSH. The signal of GSH decreased markedly in the presence of Cd^{2+} ions and the decrease of the signal could be formulated as $y = -2.0116x + 93.149$ (Figure 2B). In subsequent experiments we examined a possibility of studying the interactions with other heavy metal ions using our sensor. For this purpose Cu^{2+} , Zn^{2+} and Ni^{2+} ions were chosen. Marked changes in the GSH signal were observed (Figure 2B). We found out that

the rapid interaction between GSH and Zn^{2+} ions can be expressed as $y = -6.8433x + 99.269$. A very good interaction was observed in the case of Ni^{2+} ions ($y = -3.8932x + 100.57$) and the weakest interaction was observed in Cu^{2+} ions ($y = -0.7611x + 96.266$). By means of our proposed sensor it was possible to investigate the interaction between GSH and studied heavy metal ions below the concentration of $1\mu M$ (Figure 2B).

Interaction of glutathione with feed additive for animals

The content of thiol compounds in an organism increases with the increasing concentration of heavy metals. The aim of a feed additive in humans and animals is to benefit their health (Lichovniková and Zeman, 2004), but the effects of these compounds on physiological processes are almost unclear. Therefore we wanted to know if one of the common feed additives for animals would interact with glutathione. We added the ad-

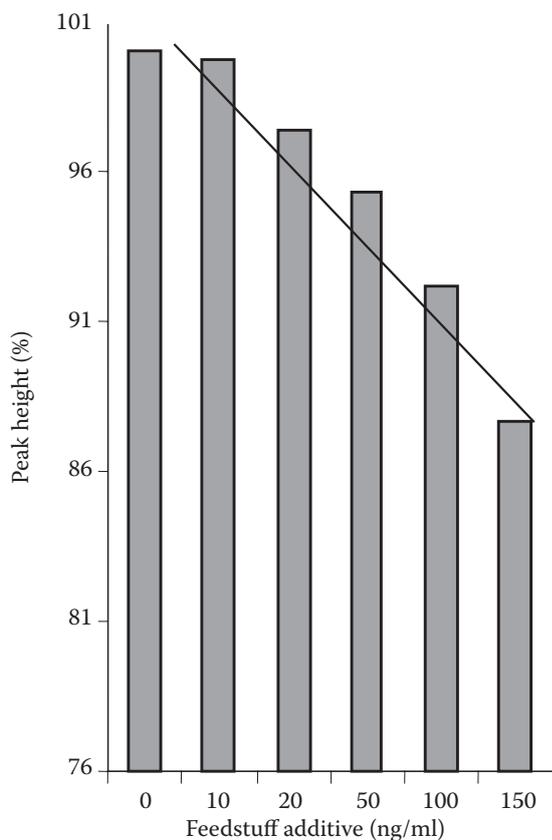


Figure 3. Influence of feed additive on the GSH signal. The peak height 100% represents 2.3 nA; for the other experimental conditions see Figure 1

ditive to GSH in the presence of supporting electrolyte. We observed that the reductive GSH signal gradually decreased (about 9%) with the increasing concentration of compounds (Figure 3). It is obvious that the proposed simple sensor can be used to study the interaction of heavy metals with feeds or fodders.

CONCLUSION

Thiols are crucial biologically active compounds with a high number of physiological functions. As we have shown, cyclic voltammetry offers a tool for studying interactions of thiols with different substances such as heavy metals and/or feed additives.

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