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Sensitive voltammetric detection of allopurinol–based drug Milurit in clinical urine samples

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Abstract

Here we summarize three electrochemical approaches to improve the sensitivity and specificity of the voltammetric sensing of purine metabolites related to the xanthine oxidase (XO) pathway (hypoxanthine-Hyp, xanthine-Xan, and uric acid-UA), as well as of their analogues used as therapeutics in the inhibition of XO enzymatic activity (allopurinol-Alo and its enzymatic product oxypurinol-Oxy) on various carbon-based materials. Detection of purine metabolites, in all proposed protocols, is based on direct electrochemical measurement of oxidation peaks for each of the monitored substances in a single detection step by the same electrode system. A nanomolar detection of these purine metabolites is possible due to: (i) mechanical roughening of the surfaces of glassy carbon or edge plane-oriented pyrolytic graphite electrodes by 15-μm silicon carbide particles (with detection limits around 20 nM), (ii) electrochemical activation of polished carbon-based materials by cycling the potential in 0.1 M KNO_3 between -0.1 and +1.85 V, and (iii) anodic stripping of the electrochemically accumulated purine-Cu(I) complexes from commonly used carbon/graphite materials in the presence of copper ions. Contrary to the mechanical roughening, both electrochemical anodization of carbon-based surfaces and anodic stripping of accumulated purine-Cu(I) complexes are less sensitive to detect Alo in the presence of Hyp due to partial overlapping of their oxidation peaks. However, all of the proposed protocols are operational to fast, sensitive, and inexpensive determination of UA, Xan as well as Oxy in a 10-μl volume of 1000 times diluted urine samples from patients treated with the Alo-based drug Milurit.

Keywords: voltammetry, carbon materials, purine catabolites, urine

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Introduction

It is important to monitor the concentration of purine metabolites in cell or body fluids to find out metabolic defects which are characterized by abnormal concentrations of the metabolites resulting in indications of pathological conditions such as gout, hyperuricaemia, Lesch-Nyan disease, renal failure, diabetes, high blood pressure, kidney disease, and heart disease (Lakhsmi et al., 2011). Our previous work has shown that differential pulse voltammetry in connection with mechanically roughened edge plane-oriented pyrolytic graphite (g-PGEe) can serve as a simple and efficient tool for monitoring transformation of purine catabolites (Hyp, Xan, and UA) catalyzed by XO as well as inhibition of this pathway by Alo being enzymatically converted to Oxy (Hason et al., 2009). In addition, mechanically roughened glassy carbon and/or edge plane-oriented pyrolytic graphite electrodes allow simultaneous detection of excreted metabolites related to the XO pathway (including relevant therapeutics) in a 1000 times diluted urine of patients treated or not treated with an Alo-based drug Milurit. This work is aimed at developing the simplest possible electrochemical approaches for improving the sensitivity and selectivity of the voltammetric monitoring of excreted purine metabolites in microlitre volumes of clinical urine samples using carbon-based materials. Stirring of the solution in the accumulation step is an important factor which contributes to amplify the oxidation signals of purine-based molecules. Continuous motion (rotation) of a 10-μl droplet of the analysed diluted urine samples was attained by inert gas streaming (bubbling) through the sample drop, which forms the recently proposed three-electrode inverted drop microcell (Hason et al. 2006; Hason et al. 2008; Hason et al. 2009a,2009b; Lakshmi et al. 2011).

Materials and Methods

Voltammetric measurements were performed using an Autolab PGStat12 potentiostat (Metrohm Autolab B.V., Utrecht, The Netherlands) connected to a three-electrode system involving different carbon/graphite as working electrodes, Ag|AgCl|3MKCl reference electrode, and a platinum wire (1-mm diameter) auxiliary electrode. All measurements were carried out at room temperature either in the classical electrochemical cell (usually in 1 ml volume) or in a homemade inverted drop microcell (usually 10 μl volume). Surfaces of an edge plane-oriented pyrolytic graphite electrode (PGEe) and glassy carbon electrode (GCE) were mechanically

roughened (g-PGEe and g-GCE) with silicon carbide paper of 1200-grit (corresponding to 15-μm abrasion particles). The GCE surface was polished (p-GCE) with 1-μm diamond paste. We also performed an electrochemical pretreatment of polished GCE (ea-GCE). The electrochemical activation was conducted with linear cyclic sweep at 50 mV s^{-1} between potentials of -0.1 V and +1.85 V in 0.1 M KNO_3 during 10 cycles. Oxidation peaks of the monitored substances were measured by means of anodic stripping differential pulse voltammetry in 0.1 M acetate buffer (pH 4.8) with the following settings: pulse amplitude of 25 mV; pulse width of 50 ms; scan rate of 15 mV s^{-1}; potential of accumulation (E_A =+0.2 V); accumulation time (t_A = 2 min); rate of stirring of the bulk solution (ω = 3000 min^{-1}); solution in the inverted drop microcell was bubbled by argon at a constant pressure of 0.04 bar. Urine samples from healthy volunteers (not dosed with Alo) and from patients suffering from gout (dosed with 100 mg or 300 mg of Milurit per day for 21 days) was collected in the Department of Internal Medicine and Hepatogastroenterology, University Hospital and Faculty of Medicine, Masaryk University.

Results and Discussion

Fig 1A shows that an equimolar mixture of purine metabolites UA, Xan, Hyp and their structural analogues Alo and Oxy (isosters of Hyp and Xan) produced well-developed and separated voltammetric signals at nanomolar concentrations at the g-PGEe. The inset of Fig 1A shows that the oxidation signals of any of these substances at the g-PGEe were not affected by any significant background current perturbations even at such low concentrations close to the detection limit. Figure 1B compares the current densities of the oxidation peaks of UA, Xan, Oxy, Hyp, and Alo (500 nM each) measured at the g-PGEe, ea-GCE, and p-GCE with a 200 μM Cu(II).

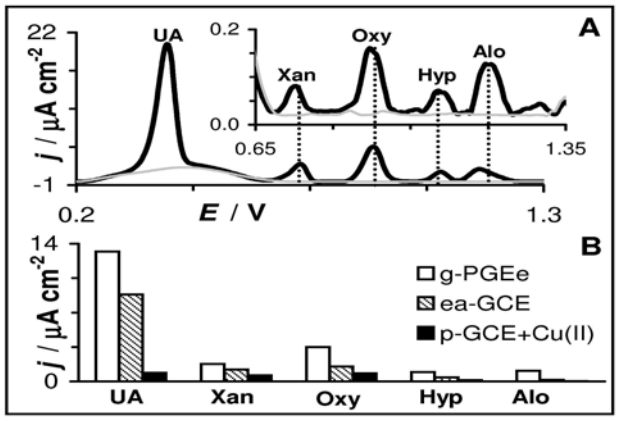

Figure 1 (A) Baseline-corrected differential pulse voltammograms (DPVs) measured for a mixture of UA + Xan + Oxy + Hyp + Alo (500 nM each). The inset shows sections of baseline-corrected DPVs measured for a mixture of UA + Xan + Oxy + Hyp + Alo close to their detection limits (50 nM each). Gray lines represent the blank electrolyte. All curves were recorded at g-PGEe. (B) Current densities of the oxidation peaks of UA, Xan, Oxy, Hyp, and Alo produced by an equimolar mixture of these five substances (500 nM each) measured at different pretreated carbon electrodes (as indicated in the Figure, for details see Experimental). The measurements were performed in the usual 1-ml voltammetric cell.

It can be seen from Fig 2 that DPV response of a 1000 times diluted urine from a healthy volunteer shows only two well-developed and separated oxidation peaks attributable to oxidation of UA (at + 0.42 V) and Xan (at +0.75 V). In the case of patients treated with the drug Milurit containing Alo as the active substance, in addition to the voltammetric peaks of UA and Xan, we can observe a new well-developed oxidation peak at +0.95 V corresponding to the electrochemical oxidation of Oxy (enzymatic product of Alo).

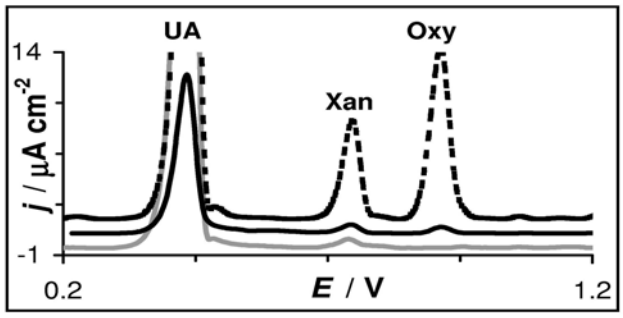

Figure 2 Baseline-corrected DPVs measured at g-PGEe for control human urine (gray curve) and for two human urines collected from patients suffering from gout and dosed with 100 mg (black full curve) and 300 mg (black dashed curve) Milurit per day for 21 days. All urine samples were diluted 1000-fold. The measurements were performed in the inverted 10-μl droplet microcell.

The use of inert gas bubbling to drive the motion of microlitre volumes (10-μl) led to a similar enhancement of oxidation signals of purine metabolites with at least 100-times-reduced sample volume compared to those obtained after accumulation from a stirred solution in the usual 1-ml electrolysis cell (Fig 2).

Conclusion

The electrochemical methods proposed show that urine excretion of the purine metabolites (UA and Xan) as well as of Oxy in patients treated with the Alo–based drug Milurit can easily be monitored electrochemically in a 10-μl drop of the only diluted urine sample in the presence of other drugs such as: antibiotics, blood pressure affecting drugs, antidiabetics, or diuretics.

Acknowledgement

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