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Sensing of sulfhydryl based compounds by a simple electrochemical approach

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ABSTRACT

We report a simple and effective electrochemical approach to determine thiolic compounds based on the reaction of sulfhydryl group with *o*-benzoquinone, which was freshly prepared by enzymatic oxidation of catechol in the presence of tyrosinase. The electrochemical reduction of *o*-benzoquinone was used as detection method by performing differential measurements in the absence and in the presence of thiols on glassy carbon electrode. Four thiols, thiocholine, cysteine, captopril and cysteamine were tested. Spectrometric measurements showed that reaction between thiols and *o*-benzoquinone is fast and complete. Therefore, indirect detection of thiols could be realized amperometrically by monitoring the decrease of the reduction current of the *o*-benzoquinone. A sensitivity of 76 and 177 μA/mM cm^2 was achieved for thiocholine and cysteine, respectively. Estimation of the total content of the compounds with sulfhydryl groups could be realized from samples containing thiols by using thiocholine or cysteine as reference compound. The proposed electrochemical approach could be used for selective detection of thiols compounds in the presence of amines at neutral pH.

1. Introduction

Sulfhydryl based compounds are involved in many physiological (glutathione, cysteine), environmental (thiocholine) and food degradation processes [1,2]. They are very reactive and many are toxic compounds. The sensitive detection of thiols is crucial for environmental and food quality control [3]. The detection of thiol drugs such as captopril, penicillamine, N-acetylcysteine, and their metabolites is important in both clinical and wastewaters analysis [4].

Spectrophotometric [5] and chromatographic methods [6,7] are the main approaches reported in the literature for detection of thiols. Biochemical processes based on thiol oxidation in the presence of quinones can also be used for detection of these compounds. Natural polyphenols like caffeic acid and its derivatives, which are well known as anti-oxidant products, can be oxidized to quinonic compounds, which can react reversibly or irreversibly with amino acids and thiols [8]. The reaction involves free nucleophilic functional groups such as sulfhydryl, amine or imidazole ring and leads to the formation of quinone based adducts [9]. These natural processes were exploited from analytical point of view by using catechol or derivatives in different ways [10,11]. Oxidation of catechol to 1,2-benzoquinone (*ortho*-quinone) can be performed electrochemically or enzymatically. Both compounds are electrochemically actives and present different spectral properties. Therefore, for studying of this reaction, spectrometric and electrochemical detection could be performed for both oxidation and reduced forms. For instance, the formation of a stable adduct between 1,2-benzoquinone and dimethylamine was used for spectrophotometric determination of *o*-benzoquinone (*o*-BQ) [12].

There are some reports about reaction of electrogenerated *o*-BQ with sulfides [13–15]. All are based on the electrochemical oxidation of catechol to *o*-BQ followed by the chemical reaction between *o*-BQ and thiol with formation of a catechol-thiol (CT) adduct. The analytical protocols proposed for detection of sulfhydryl based compounds are using different electrochemical approaches: one based on electrochemical oxidation of the CT adduct to a quinone-thiol (QT) adduct [14], and the second one based on accumulation of the QT adduct on the electrode surface and electrochemical reduction of this adduct using DPV [11] or SWV technique [15]. First approach based on the increase of the oxidation current of catechol due to the presence of the CT adduct requires degassing with argon prior each measurement [14]. The increase of the

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current attributed to the adduct re-oxidation was used to quantify the cysteine in biological media. Experiments based on electroreduction were realized at pH = 2 when accumulation of the QT adduct on the electrode surface occurs. To increase the sensitivity up to six consecutive deposition-measurements cycles [15] or up to 20 scans in cyclic voltammetry [11] were used. These approaches have several disadvantages. The electro-oxidation of catechol could lead not only to the formation of *o*-BQ but also to some *o*-BQ-based polymers that are deposited on the electrode. Moreover, the accumulation of the QT at the electrode surface can also block the surface and require a supplementary polishing step or other treatment before each measurement. Also, the very low working pH could have negative effect for determinations on real samples.

Detection of captopril, a drug with sulfhydryl group, by electrochemical oxidation of catechol produced in the reaction between the drug and quinone was also reported [16]. The disadvantage of this approach is that catechol could be oxidized more easily in presence of oxygen and the results could be affected by negative errors. The electrochemical oxidation of the catechol has also the disadvantage of accumulation of the quinone at the electrode surface and fouling of the electrode. Sensitive detection of captopril in biological samples was realized by using a catechol-derivative compound and CNT modified electrode with a detection limit of 0.034 μM [17]. Interferences from other thiols were not studied.

Cysteine detection was performed by reduction of the quinone-cysteine adduct obtained in a sequence of electrochemical–chemical–electrochemical reactions [15]. Anyway there is no proof that the adduct detection is selective toward *o*-quinone. Square wave voltammetry was used for the detection of cysteine by reduction of its adduct with 2,3-dimethoxy-5-methyl-1,4-benzoquinone but the reduction peak could be distinguished only at pH 3 or lower [18].

Biosensors based on immobilized tyrosinase are also reported for detection of L-cysteine by using the thiol–quinone interaction [19]. In order to monitor the reaction between catechol and L-cysteine in the presence of other thiolic compounds, differential measurements in the presence and in the absence of the enzyme has to be performed. A potentiometric detection of thiols was performed by using an equimolecular mixture of benzoquinone/hydroquinone [13]. The modification of the ratio between the components of the redox couple was correlated with the amount of thiol in the sample. Sensitivity and detection limit are dependent on the concentration of redox species. A response range for no more than one decade of concentration was achieved for glutathione.

In order to overcome the disadvantages of already reported systems we proposed a different electrochemical approach for studying the reaction between *o*-BQ with thiols. The polymerization of *o*-BQ and fouling of the electrode surface by this compound was avoided by oxidizing catechol enzymatically. The working principle is presented in Fig. 1. The decrease of the amount of *o*-BQ due to the consumption in the reaction with thiolic compounds is used to quantify these compounds. Differential pulse voltammetry and amperometry were used to detect *o*-BQ in the absence and in the presence of the thiol.

2. Experimental

2.1. Materials and reagents

L-Cysteine, cysteamine, captopril ((2*S*)-1-[(2*S*)-2-methyl-3-sulfanylpropanoyl] pyrrolidine-2-carboxylic acid), glycine, acetylthiocholine chloride (ATCh, purity ≥99%), catechol (purity ≥99%), tyrosinase (E.C. 1.14.18.1, from mushroom, 1715 IU/mg), acetylcholinesterase (AChE, E.C. 3.1.1.7, from electric eel, 425.94 IU/mg), potassium chloride, sodium phosphate dibasic, potassium phosphate monobasic were purchased from Sigma–Aldrich, L-glutamic acid from Merck, L-arginine and L-histidine from Fluka. All the solutions were prepared in ultrapure water (MilliQ, 18.2 MΩ cm). The AChE solution was prepared in 0.1 M phosphate buffer (PBS), pH 8, and the tyrosinase solutions were prepared in 0.1 M PBS, pH 7. Thiocholine 0.1 M stock solution was prepared daily from a ATCh stock solution 0.1 M by enzymatic hydrolysis in the presence of 1 IU AChE, for 30 min.

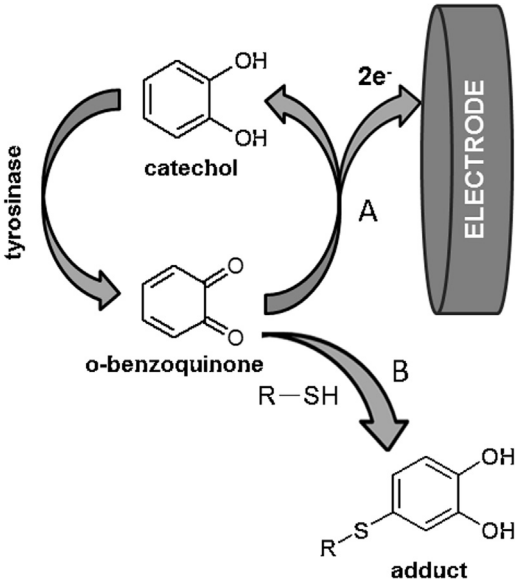

Fig. 1. Schematic representation of the electrochemical detection of thiolic compounds. A – the electrochemical reduction of *o*-benzoquinone to catechol; B – the reaction between *o*-benzoquinone and thiolic compound.

The electrochemical measurements were carried out in PBS, pH 7. The *o*-benzoquinone solutions were prepared by oxidizing catechol enzymatically. The incubation time and the amount of enzyme were optimized spectrometrically (see Section 3.1). Being relatively unstable in aqueous solution, fresh solutions of *o*-benzoquinone were prepared for each set of experiments [20].

2.2. Instruments

Cyclic voltammetry (CV), differential pulse voltammetry, amperometry and electrochemical impedance spectroscopy (EIS) measurements were performed using an Autolab PGSTAT12 potentiostat/galvanostat with FRA2 module (Eco Chemie B.V., Utrecht, The Netherlands). The GPES 4.9 and FRA software was used to collect the experimental data. A three-electrode system was used with a glassy carbon (3 mm diameter) as a working electrode, Ag/AgCl electrode 3 M KCl as the reference electrode and a Pt wire as the auxiliary electrode (Metrohm AG, Herisau, Switzerland). The glassy carbon electrode was polished using alumina slurry with particle size of 0.3 μm and 0.05 μm. All experiments were carried out at the room temperature, using a 10 mL electrochemical cell. The spectrophotometric measurements were carried out with an UV–Vis Varian Cary 100 Bio spectrophotometer using 1 cm quartz cuvette. A Varian dual cell Peltier accessory with stirring speed control attached to the spectrophotometer was used for homogenization of the solution inside the cuvette.