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Quantitative determination of biogenic amine at gold in the presence of secondary amine during electrochemical oxidation in physiological solution containing ascorbic and uric acids

Teresa Łuczak*, Maria Bełtowska-Brzezinska

Faculty of Chemistry, A. Mickiewicz University, Umultowska 89 b, PL-61-614 Poznań, Poland

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ABSTRACT

Electrooxidation of epinephrine (EP) in the presence of secondary amine as a nucleophile was investigated on a bare polycrystalline gold electrode in a phosphate buffer solution of pH 7. The results have shown that electrochemically produced epinephrinequinone underwent an attack by morpholine (MO) via 1,4-Michael addition. The reaction products were identified by electrospray ionisation mass spectrometry (ESI-MS). A linear relationship between the current response and epinephrine concentration in the presence of morpholine was obtained in the range of 2×10^{-5} mM to 0.7 mM with the detection limit 1.5×10^{-5} mM. The procedure of using morpholine as an additive in analysed solutions was proven to be suitable for quantitative epinephrine determination in samples containing an excess of the ascorbic acid (AA) and uric acids (UA) without the necessity of any preceding modification of a gold electrode surface.

1. Introduction

Catecholamine neurotransmitters are an important group of biogenic compounds which act as mediators in transmitting messages between neurons. From among neurotransmitters epinephrine is of particular importance because it plays an essential role in functioning of the mammalian central nervous and cardiovascular systems [1–3]. Therefore, one of the main tasks of the current neurochemistry is development of a simple and quick method for epinephrine detection and determination.

Catecholamines are easily oxidised, thus electrochemical methods appear to be suitable for their quantitative detection [2]. However, their oxidation products transform into polymeric compounds and poison the electrode surfaces. Moreover, catecholamines exist in natural environment together with some biomolecules like ascorbic acid (AA) and uric acid (UA), which undergo electrooxidation in an almost the same potential range [4–9]. Among various attempts to overcome the above-mentioned problems, much attention has been paid to application of chemically or electrochemically modified electrode surfaces. For example glassy carbon electrodes modified by osmium and cobalt hexacyanoferrate [6,10–12], 5-amino-1,3,4-thiadiazole-2-thiol [13], polycaffeic acid [9], luminol [14], polyrutine [15] or covered by overoxidised polypyrrole film [10], pyrolytic graphite electrode modified with carbon nanotubes [16] or impregnated with paraffin [17] as well as mesoporous Al-incorporated SiO_2 modified electrode [18] have been tested for determination of trace amounts of EP with elimination of the AA interference. Furthermore, a selective EP detection in the presence of AA and UA was reported for carbon fibre electrodes covered with overoxidised polypyrrole and DNA [19], for glassy carbon electrode modified with gold nanoparticles [20,21] or Zn–Al hydroxide film [22] and for gold electrodes covered with an overoxidised epinephrine film [23] as well as for multi-walled carbon nanotube modified with cobalt phtalocyanine [24]. The electrochemical behaviour of EP has been also studied at cysteine [25,26] SAM modified gold electrodes and at the gold nanoparticles assembled on S-containing organic acids SAM layers [27–30].

Until now, no publications concerning the electroanalytical determination of epinephrine on unmodified electrodes have been found in literature albeit it would be of great interest to simplify a detection procedure as well as the preparation mode of the respective sensor. In the present work, following this idea, the electrochemical reactivity of epinephrine in the presence of morpholine as a nucleophile was studied at a bare gold electrode in a phosphate buffer at pH 7. It is well known that morpholine easily undergoes addition to epinephrine derivatives via the intermolecular 1,4-Michael reaction [31,32] which was earlier recognised for o-benzoquinones formed upon catechol oxidation on carbon electrodes after addition of nucleophiles such as aliphatic amines

* Corresponding author. Tel.: +48 61 8658008; fax: +48 61 8658008.
E-mail address: telucz@amu.edu.pl (T. Łuczak).

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[33,34], barbituric and benzenosulfinic acid [35,36], cyanide ion [37] or aminobenzenesulfonate [38]. This may be a way to prevent the intramolecular cyclisation of epinephrinequinone to leucoepinephrinechrome and thus formation of epinephrinechrome upon epinephrine oxidation. Consequently, poisoning of the electrode surface with the strongly adsorbed species should be avoided after addition of morpholine to the analysed samples. For possible development of an efficient epinephrine sensor it is of fundamental importance to recognise the electrochemical behaviour of this compound in the presence of morpholine in samples containing UA or AA.

2. Experimental

2.1. Reagents

All chemicals were analytical grade quality and were used as obtained without further purification. The solutions were prepared from deionized water purified in a Millipore Milli-Q system, epinephrine, morpholine and ascorbic acid from Fluka, NaH_2PO_4, K_2HPO_4 and NaOH from Merck. In this work, 60 mM phosphate buffer solution of pH 7.0 was used as a supporting electrolyte. All solutions under investigation were deaerated by highly pure argon. A continuous flow of argon was maintained over the solution during the experiment.

2.2. Apparatus

The voltammetric measurements were performed in a three-compartment cell separated by glass frits using an Autolab potentiostat/galvanostat analyzer (Eco Chemie, B.V., Utrecht, The Netherlands). The pH of the solutions investigated was measured using a pH-meter (Model-ULAB 2002, TELE-ECO-PROJECT, Poland).

The working electrode was a bare gold electrode in the shape of a cylinder (0.5 mm diameter and 12 mm length). A gold sheet and a saturated calomel electrode (SCE) were used as auxiliary and reference electrodes, respectively. The gold (purity 99.999%) electrodes and the SCE electrode were purchased from the Polish State Mint and EuroSensor, Poland, respectively. Before use, the bare gold electrode was polished with aluminium slurries of successively decreasing final grades (down to 0.05 μm, Buehler) on polishing cloths (Buehler). Then it was sequentially rinsed in acetone and water. After rinsing with water the electrode was electrochemically activated by cycling at the scan rate of $v = 0.1\ V\ s^{-1}$ in the potential range between $E = -0.8$ V and $E = 0.6$ V versus SCE in 60 mM phosphate buffer (pH 7) until a stable cyclic voltammogram (j–E curve) was obtained. This procedure is known to protect the surface of gold electrodes against structural changes [39].

Electrospray mass spectra (ESI-MS) were recorded on ZQ Waters & Micromass Mass Spectrometer (Manchester, UK) with quadrupole analyser. The solution studied was introduced to the ionisation source (393 K) at a flow rate of 40 μl min^{-1} through a Harvard's pump. Nitrogen was used as the spraying gas (100 l h^{-1}) and as the drying gas (300 l h^{-1}). The electrospray capillary voltage was set to 3 kV and the extractor was set to 4 V. A cone voltage of 30 V was chosen. ESI mass spectra were acquired in MCA (multi channel acquisition) mode.

3. Results and discussion

3.1. Oxidation of epinephrine alone at a gold electrode surface

Exemplary cyclic voltammograms (CVs) of a gold electrode in the supporting electrolyte, 60 mM phosphate buffer solution (pH 7.00), containing 0.5 mM epinephrine (EP) and in the supporting

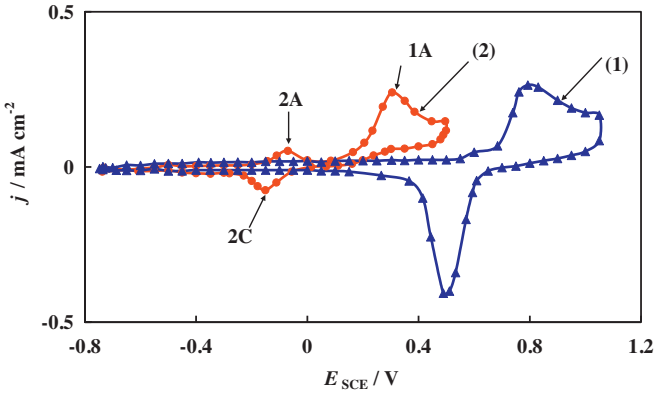

Fig. 1. Cyclic voltammograms (5th scan) of a bare gold electrode (1) in phosphate buffer solution of pH 7 (▲) and (2) with epinephrine of 0.5 mM (●). $v = 0.1\ V\ s^{-1}$.

electrolyte solution alone under the same experimental condition, recorded after the 5th scan are presented in Fig. 1. As can be seen, no electrochemical reactions take place on a bare gold electrode in the base electrolyte solution in a wide potential range between $E \approx -0.75$ V and $E \approx 0.55$ V vs. SCE (CV 1). It is well known that the anodic peak at $E \approx 0.80$ V vs SCE and cathodic peak at $E \approx 0.50$ V vs SCE correspond to the gold oxide formation and to its reduction, respectively [40]. After addition of epinephrine into the solution electrooxidation of this compound proceeds in two potential ranges. The anodic peak (1A) with a maximum at $E = 0.31$ V vs SCE (CV 2) having no cathodic counterpart can be assigned to the irreversible epinephrine oxidation to o-epinephrinequinone, see Scheme 1 [28–30]. Since the pK_a of EP is equal to 9.9 [28], at pH < 9.9 epinephrine exists mainly in its protonated form which shows weak nucleophilic activity for intramolecular 1,4-Michael addition. However, even in solution of pH 7 at appropriately selected epinephrine concentration the amount of unprotonated epinephrinequinone appears to be sufficient to allow the intramolecular cyclisation (chemical reaction) to take place [31]. The following electrooxidation of the resulting leucoepinephrinechrome to epinechrome during the successive potential scans and its regeneration after reversal of the scan direction are manifested in CVs by the presence of the new anodic peak at −0.07 V vs SCE (2A), accompanied by the cathodic peak at −0.13 V vs SCE (2C).

As reported earlier for gold electrodes modified with S-functionalized compounds and gold nanoparticles [28–30], the behaviour of epinephrine on a bare gold electrode, in the potential range of peak 1A was found to be dependent on pH. The peak potential ($E^{p}_{(1A)}$) was shifted in the negative direction with increasing pH of solution by about 0.059 ± 0.002 V [41], confirming that the same number of electrons and protons are released from the oxidised species. As commonly accepted [28–30], each electrochemical reaction occurring in the system investigated is a two-proton and two-electron process (Scheme 1). Similarly as at the glassy carbon electrode [10], we have observed that the cyclisation rate of epinephrinequinone becomes nearly independent of the H^+ concentration in solution between pH 6 and pH 8 but it decreases remarkably upon increasing the solution acidity. On the other hand, the intramolecular cyclisation of unprotonated qinones is favoured in solution of high pH values [11].

We have established that the maximum current density ($j_{p(1A)}$) related to epinephrine oxidation at a gold electrode increases linearly with the square root of the scan rate as typical for the electron transfer process controlled by diffusion [28–30,41]. Accordingly, a linear increase in the maximum current density with increasing substrate concentration was observed in a wide range between