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Determination of Dopamine and Uric Acid under Coexistence of Ascorbic Acid at Glassy Carbon Electrodes Pre-Treated in Adenosine Monophosphate Solutions

XIAO-HONG ZHU

Department of Chemistry and Chemical Engineering, Hefei Normal University, Hefei 230601, P.R. China

Corresponding author: Tel: +86 551 3674145; E-mail: zhuxh0802@163.com

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Glassy carbon electrodes modified with adenosine monophosphate (AMP) were described. It was fabricated by electrochemical grafting the products of the electrochemical oxidation of adenosine monophosphate at 1.8 V (*vs*. SCE) on glassy carbon electrode (denoted as AMP/GCE). The modified electrode was applied to the electrocatalytic oxidation of dopamine, uric acid and ascorbic acid and resolved the overlapping of the anodic peaks of dopamine, uric acid and ascorbic acid into three well-defined voltammetric peaks in cyclic voltammetry or different pulse voltammetry. In the presence of 1.0 mM ascorbic acid, a linear range of 0.086-21 μM with a detection limit of 0.028 μM for dopamine and in the range of 7.2-140 μM with a detection limit of 0.61 μM for uric acid were obtained. The modified electrode shows excellent selectivity and good sensitivity, antifouling properties, which was attributed to the formation of highly conjugated oxidation product bearing quinone structure during the modification process.

Key Words: Adenosine monophosphate, Electrochemistry, Sensor.

INTRODUCTION

Dopamine (DA) play very important roles in keeping the functions of human central nervous. As well known, the lack of dopamine may lead to Parkinson's disease[1]. Uric acid is the main final product of purine metabolism in humans, which is commonly regarded as an indicator of gout, epidemiological studies[2,3]. High uric acid levels in serum represent a risk factor for cardiovascular diseases[4], uric acid stones[5] and Lesch-Nyhan syndrome[6]. Therefore, many researchers have been attracted to work on the determination of dopamine and uric acid[7-10]. Electrochemical detection is one of the most simple and rapid methods. However, ascorbic acid, dopamine and uric acid usually coexist in real systems. To develop a sensitive and selective method for their simultaneous determination is of critical importance not only in the field of biomedical chemistry and neurochemistry but also for diagnostic and pathological research. Electrochemically individual and/or simultaneous determinations of them on conventional bare electrodes are difficult since they oxidized at very close potentials to result in overlapping voltammetric responses and their oxidation products very often foul the electrodes. In order to resolve this problem, a variety of chemically modified electrodes have been employed[11-13]. Electrochemical sensors based on deoxyribonucleic acid (DNA) have been reported for simultaneous determination of dopamine and uric acid in the presence of ascorbic acid[14]. Adenine, as a basic stuff of DNA, combines with the sugar ribose to form adenosine, which in turn can be bonded with from one to three phosphoric acid units, yielding the three nucleotides adenosine monophosphate (AMP), adenosine diphosphate (ADP) and adenosine triphosphate (ATP). The adenine moiety in different nucleotides, *i.e*., adenosine, AMP, ADP and ATP can be oxidized on pyrolytic graphite electrode (PGE)[15]. Adenine-derivative modified electrodes show strong electrocatalytic activity for the oxidation of the reduced form of β-nicotinamide adenine dinucleotide (NADH)[16,17], but no further studies have been done to evaluate if the modified electrodes could be used to avoid poisoning electrode effects and/or to promote catalytic oxidation of important biological molecules, such as dopamine and uric acid. Therefore, in this paper we describe the electroxidation of dopamine, uric acid and ascorbic acid using glassy carbon electrode (GCE) pre-treated in adenosine monophosphate solutions at 1.8 V(denoted as AMP/GCE). It was found that the AMP/GCE not only exhibited strong catalytic activity toward the oxidation of uric acid, dopamine and ascorbic acid but also resolved their voltammetric responses into three well-defined peaks. Hence, it could be used as an electrochemical probe for uric acid and dopamine.

EXPERIMENTAL

Adenosine monophosphate, uric acid and ascorbic acid were from Chemical Reagent Company of Shanghai. Dopamine

was purchased from Sigma. They were used as received and without further purification. All other chemicals were of analytical grade. The solutions were freshly prepared in 0.1 M phosphate buffer solution (PBS) containing 0.1 M KCl and were thoroughly deoxygenated by bubbling highly purified nitrogen before use. To mimic biological environments, pH 7.0 PBS was selected as a supporting electrolyte unless stated otherwise. All experiments were made at room temperature.

Cyclic voltammetry (CV) and differential pulse voltammetry (DPV) were performed with a CHI 832 electrochemical analyzer (Cheng-Hua, Shanghai, China). All electrochemical experiments were carried out using a three-electrode system consisted of a working electrode, a platinum wire au xiliary electrode and a saturated calomel reference elect rode (SCE). XPS measurement was performed on an ESCALAB MK2 spectrometer (VG, UK) with an Mg K-Alpha X-ray radiation as the source for excitation.

Electrode preparation: Prior to modification, the bare glassy carbon electrode (GCE, Φ 4 mm) was successively polished with 1.0, 0.3, 0.05 μM alumina slurry and then ultrasonic cleaned in ethanol and doubly distilled water for 5 min, respectively. The finally cleaned GCE was directly immersed in $5\text{-}10^{-4}$ mol/L adenosine monophosphate solution and held at +1.8 V (*vs*. SCE) for 0.5 h to fabricate adenosine monophosphate modified electrode (AMP /GCE). After rinsed with distilled water, the physical adsorbed molecules were removed by ultrasonication in PBS solution for 5 min and then AMP/GCE was stored in 0.1 M PBS-KCl ready for use.

RESULTS AND DISCUSSION

Characterization of AMP/GCE: It was discussed that the cyclic voltammetry of AMP at the pyrolytic graphite, in which the AMP molecules could also be oxidized and proceed in two parallel routes of electron transfer reactions resulting in the formation of a variety of O-O-, C-C- and -O- linked dimers[15]. Otherwise, some amine containing compounds could firmly link on the surface of carbon electrodes by the formation of C-N bound during electrochemical oxidation[18], it is also possible that the AMP molecules may be oxidized forming C-N linkage to the activated carbon surface too. In this report, the adenosine monophosphate molecule was successfully immobilized from water solutions onto GCE under controlled dc potentials for study. The AMP molecule could also be oxidized rapidly giving a molecule with a quinone . diimine structure at potentials higher than about 1.3 V, the application of 1.8 V electrode potential would ensure that the GCE could be well activated to cation radicals, which then react with the oxidation products of AMP molecules at -NH sites, generating C-N linkages for surface grafting.

The covalent linkages on the GCE surface were demonstrated by XPS elemental analysis. Fig. 1 shows the XPS spectrum of the AMP/GCE. Fig. 1a shows a characteristic N1s band at 400.06 eV in agreement with C-N band indicating the existence of adenosine monophosphate molecule residues grafted at the surface. The existence of P2p band at 133.7 eV demonstrated the deposition of adenosine monophosphate molecule on the electrode (Fig. 1b).

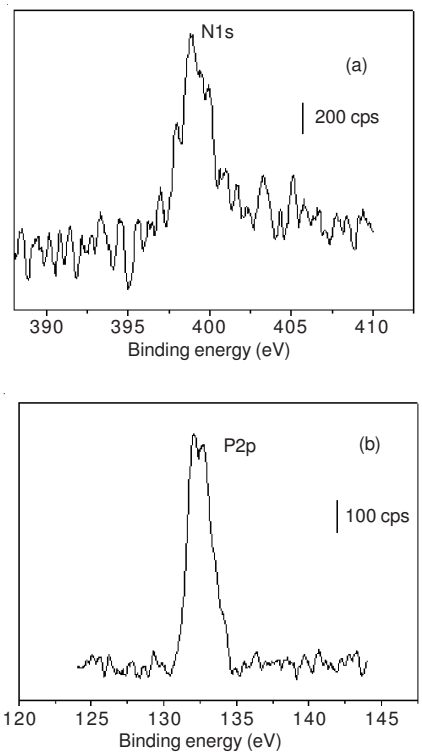

Fig. 1. XPS spectra of N1s (a) and P2p (b) for AMP /GCE

Electrochemical behaviour of dopamine, uric acid and ascorbic acid: The cyclic voltammetric curves of dopamine, uric acid and ascorbic acid at a bare GCE and the AMP/GCE are presented in Fig. 2. In the mixed solution, AMP/GCE could also give well resolved anodic peaks at 0.019, 0.163 and 0.301 V for ascorbic acid, dopamine and uric acid, respectively. In contrast, only a rather broad anodic peak can be seen at about 0.50 V at bare GCE. The negative shifts of anodic peak potentials for dopamine, uric acid and ascorbic acid indicate the AMP/GCE has strong catalytic activities toward all these oxidation reactions. The AMP molecule was oxidized rapidly to form highly conjugated oxidation product bearing quinone structure, which facilitated the electron transfer process at the interface and might be responsible for the catalytic effect observed over the oxidation of them. There may be some reasons considered for the separation of dopamine, uric acid and ascorbic acid oxidation peaks at the AMP /GCE. The apparent overpotential of ascorbic acid oxidation decreases sharply at the AMP/GCE, resulting in a significant negative shift of the anodic current peak. Moreover, due to the improvement in their reversibility of electron transfer (et) process, the dopamine, uric acid and ascorbic acid oxidation peaks are significantly less broad. Due to much higher adsorption of dopamine, uric acid at the electrode surface, dopamine and uric acid can significantly accumulate on the surface of the AMP/GCE and ascorbic acid could not. All of them provided a base for simultaneous determination of dopamine and uric acid in the presence of ascorbic acid.

Effect of scan rate: The effect of scan rate on the peak current of dopamine and uric acid at the AMP /GCE were investigated. As shown in Fig. 3a, the peak current (i_{pa}, i_{pc}) of dopamine was linearly proportional to the scan rate between 20 and 300 mV/s. The i_{pa}/i_{pc} ratio remained almost equal to

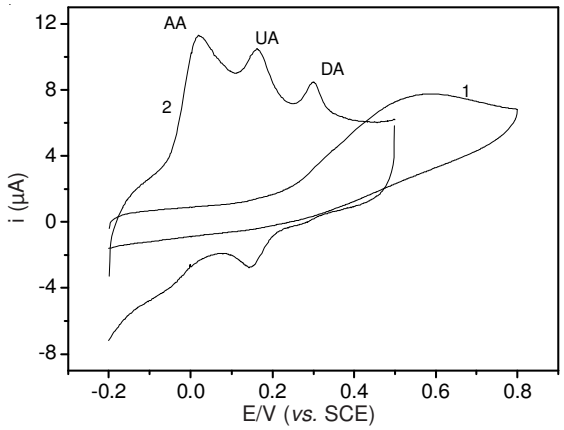

Fig. 2. Cyclic voltammetry curves of (1) bare GCE, (2) AMP/GCE in 10 μM dopamine + 10 μM uric acid + 1 mM ascorbic acid (0.1 M PBS, pH 7.0). Scan rate: 50 mV/s

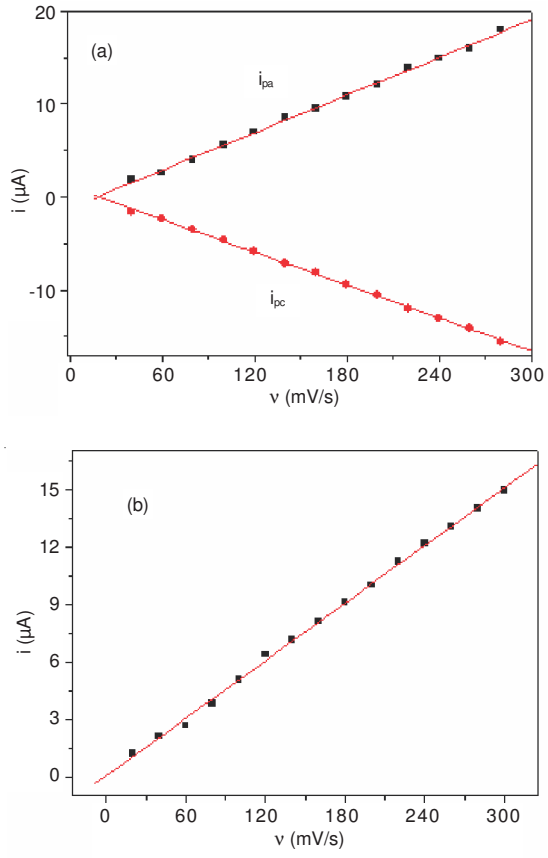

Fig. 3. Plots of (1) anodic and (2) cathodic peak current of (a) 10 μM dopamine, (b) 10 μM uric acid *versus* the scan rate at AMP /GCE

unity, as expected for a surface-type behaviour. The anodic peak current of dopamine was linear with the scan rate and the linear regression equation could be expressed as i_{pa} = -1.194 + 0.068ν with a correlation coefficient of 0.999, indicating a absorption-controlled process. According to Fig. 3b, the ipa of uric acid at AMP/GCE was proportional to the scan rate over the range of 20.300 mV. The linear regression equation was i_{pa} = 0.054 + 0.051ν with a correlation coefficient of 0.999, suggesting a absorption-controlled process. Since dopamine (pK = 8.92) and uric acid (pK = 5.4) exists as cations and anions in pH 7.0 PBS, respectively, dopamine and uric acid should be accumulated on the electrode surface from solutions due to molecule interactions with the modified layer. As a result of phosphate groups in the modified layer, the surface of the AMP/GCE is negatively charged, which could strongly attract dopamine cations in the neutral environment. Otherwise, uric acid is one product of purine metabolites, has a structure similar to purine. The similar molecular structure may bring easy attractive interactions, in result of accumulation of uric acid molecules and improvement in its reversibility of electron transfer (et) process. However, no significant time dependent phenomenon was observed because the adsorption of dopamine and uric acid process was probably very fast on the surface. Solution switching experiments also demonstrated that a memory effect exist ed for dopamine and uric acid at the AMP/GCE.

Effect of pH: Fig. 4 shows the effect of solution pH on the response of dopamine (a) and uric acid (b) oxidation at AMP/GCE, respectively. The DPV peak potential of dopamine oxidation shifted negatively at a slope of -54 mV per pH with pH value increasing from 2.0-8.0 (Fig. 4a). According to Fig. 4b, the anodic peak potential of uric acid decreased linearly with an increase of pH from 2.0-8.0 at a slope of -60 mV per pH. These are very close to the theoretical value of -59 mV per pH for equal electrons and protons involved in the oxidation process, indicating a 2e/2H$^+$ reaction.

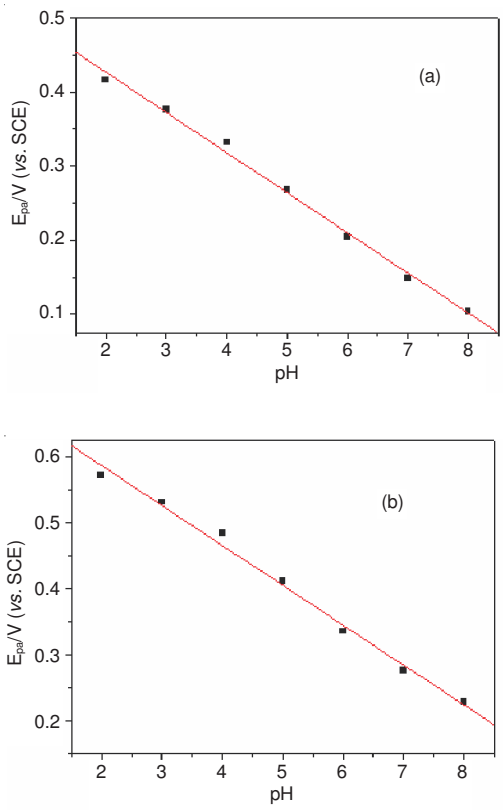

Fig. 4. Effect of pH on Epa for dopamine (a) and uric acid (b) at AMP/GCE. DPV: Scan rate: 50 mV s^{-1}, Amplitude: 50 mV, pulse width: 50 ms, pulse period: 200 ms

For comparison, the changes in dopamine and uric acid peak currents with pH were also investigated. The oxidation peak current of dopamine gradually increased with increasing pH and reached maximal values at pH 6. The peak current of

uric acid was stable between pH 2 and 6 and decreased in basic pH. Therefore, it is obvious that lower pH value is favorable for dopamine and uric acid determination, however, in order to mimic the physiological environment, pH 7 was chosen for determination in this paper.

Determination of dopamine and uric acid in the presence of ascorbic acid: To obtain better resolution, the quantitative determination of dopamine and uric acid concentration in the presence of ascorbic acid at the AMP/GCE electrode was performed with DPV and the oxidation peak current was selected as the analytical signal. The DPV gave peaks at -0.012, 0.132 and 0.264 V for ascorbic acid, dopamine and uric acid, respectively, at the AMP/GCE (Fig. 5). The use of DPV did not increase peak separations, however, did obviously improve the baselines of these peaks and enlarge the current sensitivity. The dependence of the peak current on the concentration of dopamine and uric acid in presence of 1 mmol/L ascorbic acid is presented. The catalytic peak current was linearly related to dopamine and uric acid concentration in the range 8.6×10^{-8}-2.1×10^{-5} and 7.2×10^{-6}-1.4×10^{-4} mol/L with correlation coefficient of 0.998 (the inset). The practical detection limit was 2.8×10^{-8} and 6.1×10^{-7} mol/L, respectively.

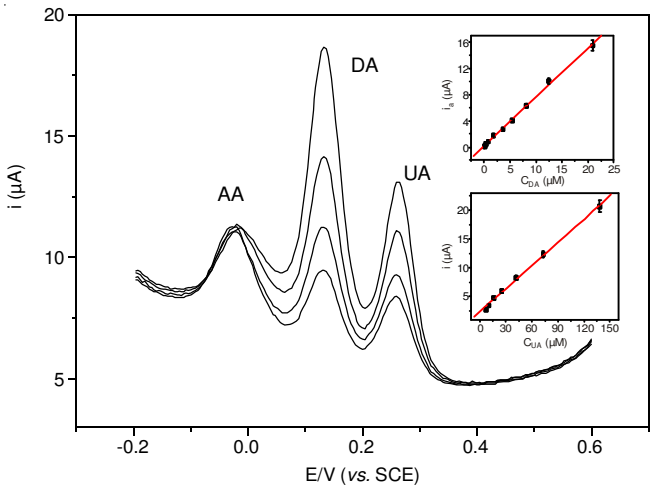

Fig. 5. DPVs of different concentrations of dopamine and uric acid at AMP/GCE in the presence of 1.0 mM ascorbic acid. Insets: the linear plots of peak current *vs.* concentration

Reproducibility and stability: Reproducibility of this electrode was tested. Because the modified polymer film could significantly adsorb dopamine, uric acid and some products of these oxidation reactions, the electrode was electrolyzed at 1 V for more than 1 min to regenerate the baseline before each determination. In this way, the memory effect on the modified film can be completely removed. It was also found that the electrode showed almost no change on the current responses after one week and only 15 % decrease after 30 d, for the storage in PBS (pH 7.0) at 4 ºC.

Conclusion

The AMP/GCE fabricated by electrochemical grafting the products of the electrochemical oxidation of AMP on glassy carbon electrode exhibits strong electro-catalytic activity toward dopamine, uric acid and ascorbic acid. Due to the formation of highly conjugated oxidation product bearing quinone structure during the modification process, this biosensor can separate the mixture of dopamine, uric acid and ascorbic acid into three well-defined DPV peak with the ΔE_{pa} of 144 mV (between dopamine and ascorbic acid) and 276 mV (between uric acid and ascorbic acid) in pH 7 PBS. Simultaneous determination of dopamine and uric acid by DPV can be well conducted in the presence of excess amount of ascorbic acid. The modified electrode has good reproducibility, selectivity and stability.

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