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Electrochemical behaviour of 2,8-dihydroxyadenine at a glassy carbon electrode

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Abstract

The electrochemical behaviour of 2,8-dihydroxyadenine (2,8-DHA)– the main adenine oxidation product– has been investigated over a wide pH range at a glassy carbon electrode (GCE) using cyclic, differential pulse and square wave voltammetry.

The oxidation of 2,8-DHA is a quasi-reversible process, pH dependent and occurs with the formation of a main oxidation product, $P_{2,8\text{-DHA}}$, that strongly adsorbs on the electrode surface. The reduction of 2,8-DHA also occurs and is a reversible process in the absence of molecular oxygen. In electrolytes with pH between 4 and 9 two consecutive reversible charge transfer reactions were identified. However, it was observed that O_2 interfered with the reductive electron transfer process of 2,8-DHA and that, in the presence of oxygen, the reduction of 2,8-DHA occurs at less negative potentials than in the absence of oxygen.

Keywords: Adenine; 2,8-dihydroxyadenine; Oxidation; Reduction; DNA; Oxidative damage; Voltammetry

1. Introduction

Purine and pyrimidine derivatives are fundamental compounds in biological systems, playing an essential role in various biological processes such as energy transduction and cell signaling [1]. In particular, the nucleotides of adenine and guanine together with those of thymine and cytosine represent the monomer units of nucleic acids. DNA is a very important biomolecule that has an essential role in the determination of hereditary characteristics, storing the genetic information. From this point of view DNA is considered the major target for interaction with various molecules.

DNA bases are modified during the interaction with various compounds such as reactive oxygen and nitrogen species (ROS and RNS), alkylating agents or drugs [2]. Chemical modification of DNA bases generates several products inside the DNA double helix such as 8-oxoguanine, 2,8-dihydroxyadenine, 5-formyluracil, 5-hydroxycytosine, etc., which are mutagenic, causing molecular disturbance to the genetic machinery that leads to cell malfunction and death [3].

The major product of DNA damage is 8-oxo-7,8-dihydroguanine (8-oxoGua) which is the product of oxidation of guanine [4], the most easily oxidized base in DNA. Since it was first reported, 8-oxoGua has been the subject of intensive investigation and became widely accepted as a biomarker of DNA damage and cellular oxidative stress [5].

However, recent studies have suggested that other lesions could be as important as 8-oxoGua [2]. It is well known that the irreversible 2 electrons and 2 protons oxidation of adenine yields 2-hydroxyadenine or 8-hydroxyadenine [6], which were also reported to have mutagenic activity [2,3]. Moreover, each of these products can also suffer irreversible oxidation leading to the formation of 2,8-dihydroxyadenine (2,8-DHA), Scheme 1, the main oxidation product of adenine [6]. Although its formation in DNA is not as efficient as the formation of 2 or 8-hydroxyadenine, the incorporation of 2,8-dihydroxy-deoxyadenosine monophosphate from the nucleotide pool appears to be the major pathway for formation of 2,8-DHA in DNA [2]. Such kinds of DNA modified bases were found in human cancerous tissues [7].

Also, 2,8-DHA is the main clinical manifestation related to the metabolic deficiency of the purine salvage enzyme adenine phosphoribosyltransferase, which converts adenine from the

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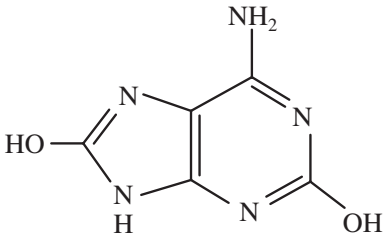

Scheme 1. Chemical structure of 2,8-DHA.

nucleotide pool into adenosine monophosphate [8]. In patients with this deficiency, the accumulated adenine is converted by xanthine dehydrogenase to 8-hydroxyadenine and then to 2,8-DHA. The precipitation and crystallization of 2,8-DHA can lead to stone formation in various parts of the urinary tract [9], leading to the loss of the kidneys.

In the view of the importance of 2,8-DHA in the biological processes various methods have been developed for the determination of 2,8-DHA in body fluids [8,10,11]. However, less is known about 2,8-DHA redox mechanisms [12]. Investigations of the redox behaviour of biologically occurring compounds by means of electrochemical techniques have the potential for providing valuable insights into biological redox reactions of these molecules. Due to their high sensitivity voltammetric methods were successfully used to study the redox behaviour of various biological compounds including the DNA bases and nucleotides as well as their oxidation products [4,12–18]. Therefore, the present study is concerned with the electron transfer properties of 2,8-DHA by means of cyclic, differential pulse and square wave voltammetry at a glassy carbon electrode.

2. Experimental

2.1. Materials and reagents

2,8-dihydroxyadenine from Sigma was used without further purification. A stock solution of 50 μM 2,8-DHA was prepared in pH 4.5 0.2 M acetate buffer and was stored at −4 °C.

All supporting electrolyte solutions, Table 1, were prepared using analytical grade reagents and purified water from a Millipore Milli-Q system (conductivity ≤ 0.1 μs cm^{-1}).

Nitrogen saturated solutions were obtained by bubbling high purity N_2 for a minimum of 10 min in the solution and continuing with a flow of pure gas over the solution during the voltammetric experiments.

Microvolumes were measured using EP-10 and EP-100 Plus Motorized Microliter Pippettes (Rainin Instrument Co. Inc., Woburn, USA). The pH measurements were carried out with a Crison micropH 2001 pH-meter with an Ingold combined glass electrode. All experiments were done at room temperature (25 ± 1 °C).

Table 1
Supporting electrolytes, 0.2 M ionic strength

pH	Composition
2.2	HCl + KCl
3.4	HAcO + NaAcO
4.5	HAcO + NaAcO
5.3	HAcO + NaAcO
6.1	$NaH_2PO_4 + Na_2HPO_4$
7	$NaH_2PO_4 + Na_2HPO_4$
8.1	$NaH_2PO_4 + Na_2HPO_4$
9.3	$NaOH + Na_2B_2O_7.10H_2O$
11.1	NaOH + KCl

2.2. Voltammetric parameters and electrochemical cells

Voltammetric experiments were carried out using a μAutolab running with GPES 4.9 Software, Eco-Chemie, Utrecht, The Netherlands. The experimental conditions for differential pulse voltammetry (DPV) were: pulse amplitude 50 mV, pulse width 70 ms, scan rate 5 mV s^{-1}. Measurements were carried out using a glassy carbon (GCE) (d = 1.5 mm) working electrode, a Pt wire counter electrode, and a Ag/AgCl (3 M KCl) as reference, in a 0.5 ml one-compartment electrochemical cell.

The GCE was polished using diamond spray (particle size 1 μm) before every electrochemical assay. After polishing, the electrode was rinsed thoroughly with Milli-Q water for 30 s; then it was sonicated for 1 min in an ultrasound bath and again rinsed with water. After this mechanical treatment, the GCE was placed in pH 4.5 0.2 M acetate buffer electrolyte and various DP voltammograms were recorded until a steady state baseline voltammogram was obtained. This procedure ensured very reproducible experimental results.

3. Results and discussion

Initial studies concerning the voltammetric behaviour of 2,8-DHA at a clean GCE were carried out in pH 4.5 0.2 M acetate buffer. The cyclic voltammogram in Fig. 1 was obtained in a solution of 50 μM 2,8-DHA saturated with N_2. During the voltammetric measurement a constant flux of N_2 was kept over

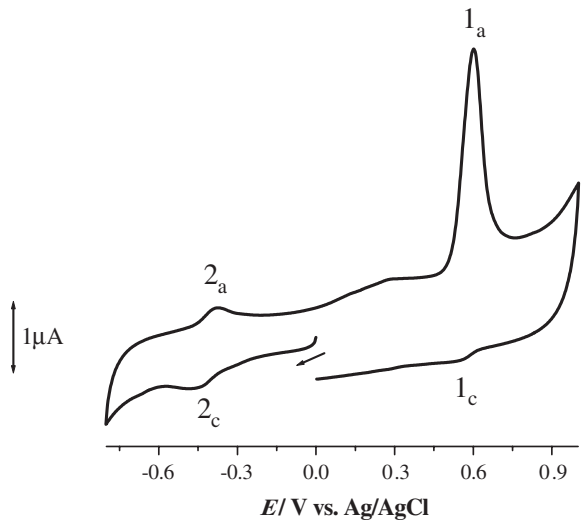

Fig. 1. CV obtained with a GCE in N_2 saturated solution of 50 μM 2,8-DHA in pH 4.5 0.2 M acetate buffer; v = 100 mV s^{-1}.

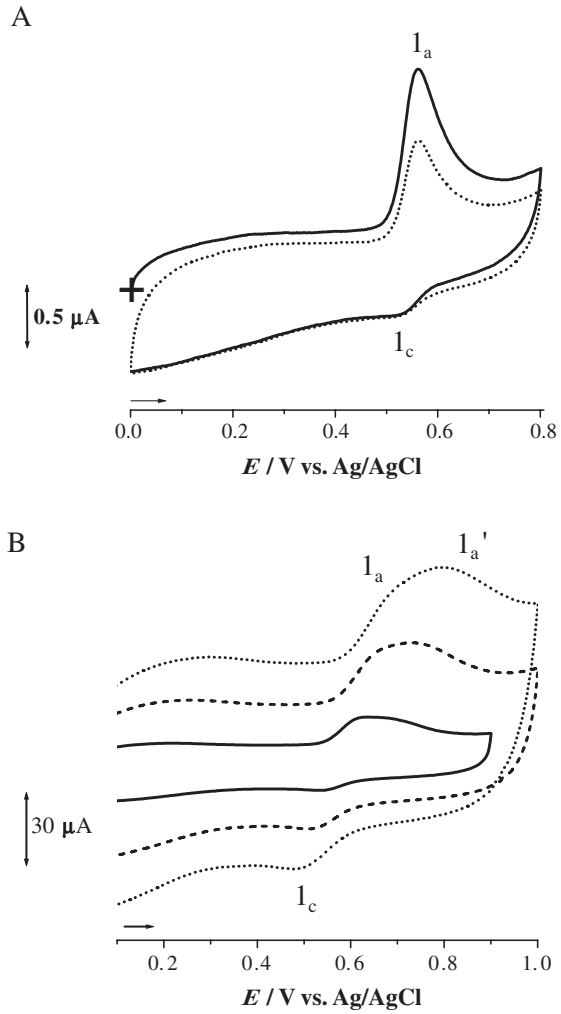

Fig. 2. CV obtained with a GCE in a solution of 50 μM 2,8-DHA in pH 4.5 0.2 M acetate buffer: A) (—) 1st and (·····) 2nd scans at $v = 50$ mV s^{-1} and B) 1st scan at (—) 1, (- - -) 5 and (·····) 10 V s^{-1}.

the solution surface in order to avoid the diffusion of atmospheric oxygen into the solution of 2,8-DHA.

Two pairs of peaks can be observed in Fig. 1. The reduction and oxidation of 2,8-DHA occur independently of each other and are going to be studied separately.

3.1. Oxidation

3.1.1. Cyclic voltammetry

The oxidation of 2,8-DHA at a GCE was investigated in pH 4.5 0.2 M acetate buffer. The CV obtained in a 50 μM 2,8-DHA solution at a scan rate $v = 50$ mV s^{-1}, Fig. 2A, shows only one main anodic peak 1_a at $E_{pa} = +0.58$ V, due to the oxidation of 2,8-DHA. On scanning in the negative direction, a reduction peak 1_c is observed at $E_{pc} = +0.52$ V. This peak 1_c corresponds to the reduction of the oxidation product of 2,8-DHA. Although the difference in potential between the anodic and the cathodic peak is about 30 mV, the peaks 1_a–1_c form a quasi-reversible couple since the ratio of their currents $I_{pa}/I_{pc} > 1$. The decrease of peak 1_a oxidation current, observed on the second CV scan, is due to the adsorption of 2,8-DHA oxidation products at the GCE surface.

CVs were also obtained for different scan rates in 50 μM 2,8-DHA in pH 4.5 0.2 M acetate buffer. From Fig. 2B it was observed that increasing the scan rate, the peak 1_a potential is displaced to more positive values. Simultaneously, the width at half-height of peak 1_a increases such that, for $v \geq 2$ V s^{-1}, peak 1_a occurs overlapped with a new peak $1'_a$. It is suggested that this new peak $1'_a$ corresponds to the oxidation of 2,8-DHA dimers formed at the GCE surface during the oxidation of 2,8-DHA molecules. The formation of such kind of dimers is well documented in the literature [15,17,18], but they can only be observed when high scan rates are used ($v \geq 2$ V s^{-1}) probably because they have a short lifetime.

At the same time, the cathodic peak 1_c is displaced to more negative values whereas its current increases with the scan rate. Nevertheless, the fact that peak 1_c current is always smaller than the peak 1_a current suggests that the oxidation product of 2,8-DHA is very unstable and undergoes hydrolysis in the solution [6].

3.1.2. pH effect; Differential pulse voltammetry

The electrochemical oxidation of 2,8-DHA was studied over a wide pH range between 1 and 13 using DPV. The DP

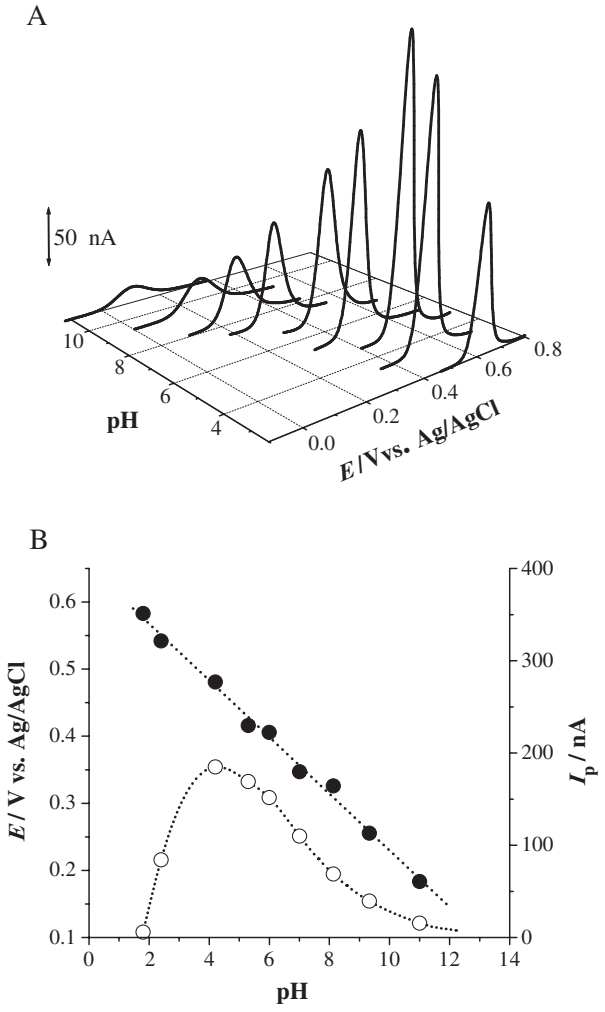

Fig. 3. A) 3D plot of DPV obtained in 50 μM 2,8-DHA as a function of pH. B) Plots of (●) E_{pa} and (○) I_{pa} of peak 1_a vs. pH.

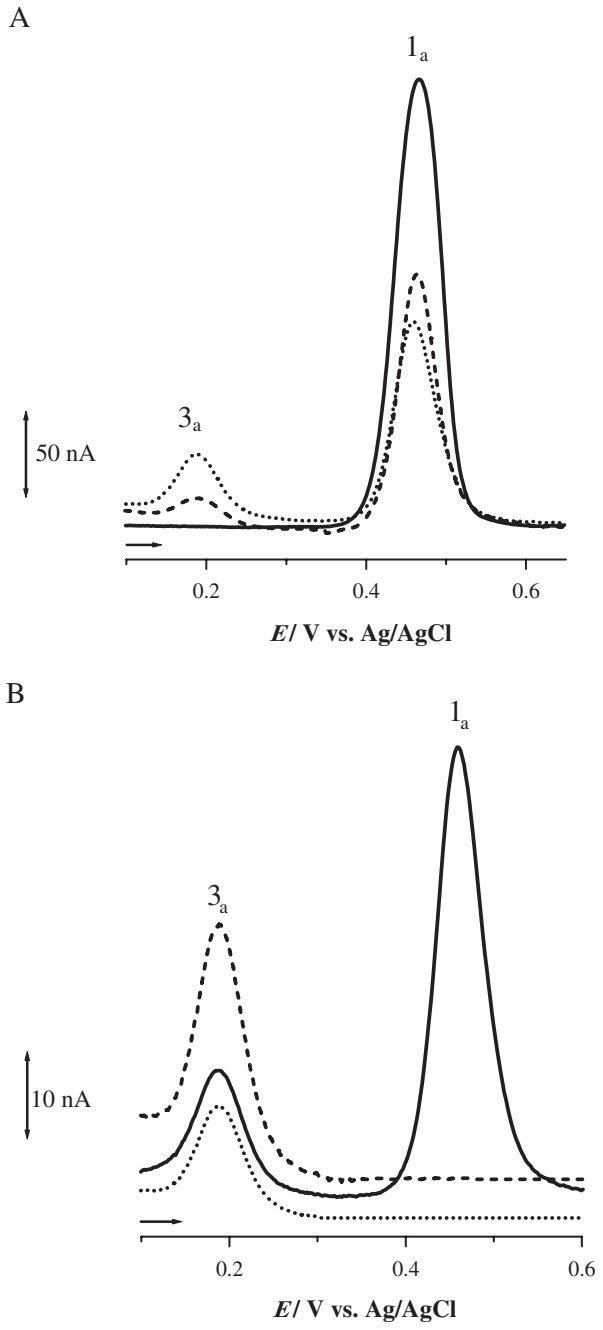

Fig. 4. Base line subtracted DPV consecutively recorded: A) in 50 μM 2,8-DHA in pH 4.5 0.2 M acetate buffer or B) after the transference of GCE from the solution of 2,8-DHA to pH 4.5 0.2 M acetate buffer; (——) 1st, (- - -) 2nd and (·····) 3rd scan.

voltammograms, Fig. 3A, were all recorded in solutions of 50 μM 2,8-DHA in different electrolytes with 0.2 M ionic strength.

The peak 1_a potential is displaced to more negative values with increasing pH. The dependence is linear over the whole pH range and follows the relationship E_{pa} (V) = 0.65–0.042 pH, Fig. 3B. The slope of the line, 42 mV per pH unit, shows that the oxidation mechanism of 2,8-DHA involves electron transfer followed by a chemical reaction, probably dimerization, leading to irreversible product adsorption at the electrode surface [19].

However, the width at half-height of peak 1_a was always $W_{1/2}$ = 49 mV, which suggests that the oxidation of 2,8-DHA occurs with the transfer of 2 electrons.

The oxidation current of 2,8-DHA increases rapidly with pH reaching a maximum in pH 4.5 0.2 M acetate buffer, Fig. 3B. For this reason this supporting electrolyte was chosen to study the electrochemical oxidation mechanism of 2,8-DHA.

Successive DP voltammograms were recorded in a solution of 50 μM 2,8-DHA for pH 4.5 0.2 M acetate buffer, Fig. 4A. The oxidation of 2,8-DHA, peak 1_a, occurs at E_{pa} = +0.47 V. On a secondary DP scan, a new peak 3_a occurs at E_{pa} = +0.19 V, and its amplitude increases with the number of potential scans. This peak corresponds to the oxidation of the product of oxidation of 2,8-DHA, $P_{2,8\text{-DHA}}$, which is strongly adsorbed on the electrode surface. At the same time, the oxidation current of 2,8-DHA decreases gradually with the number of potential scans due to the decrease of the available electrode surface because of the adsorption of $P_{2,8\text{-DHA}}$.

The adsorption of $P_{2,8\text{-DHA}}$ at the GCE surface was confirmed when, at the end of several DP scans recorded in

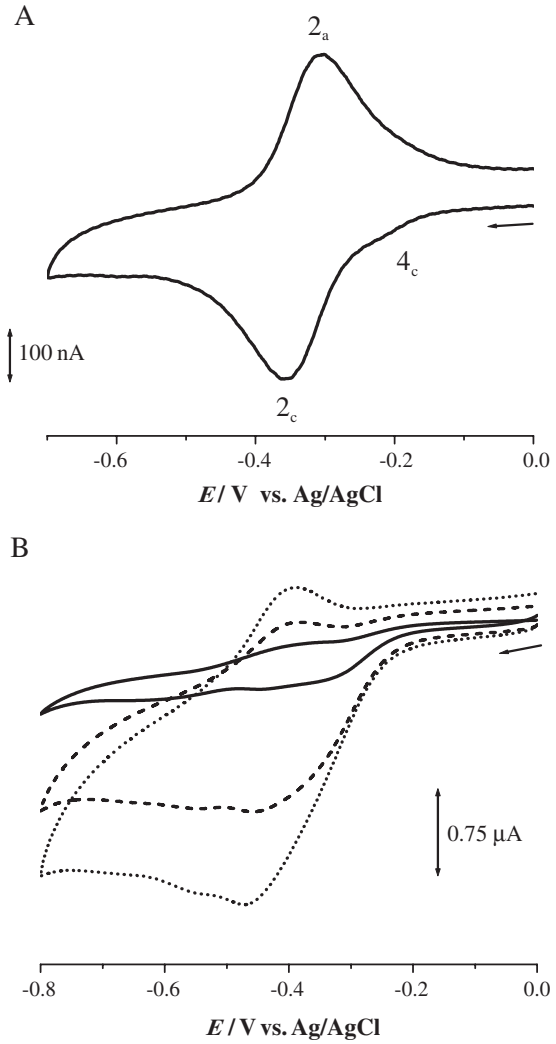

Fig. 5. CV obtained with a GCE in solutions of 25 μM 2,8-DHA in pH 4.5 0.2 M acetate buffer: A) saturated with N_2; v = 50 mV s^{-1} or B) normal atmosphere; scan rates: (——) 10, (- - -) 50 and (·····) 100 mV s^{-1}.

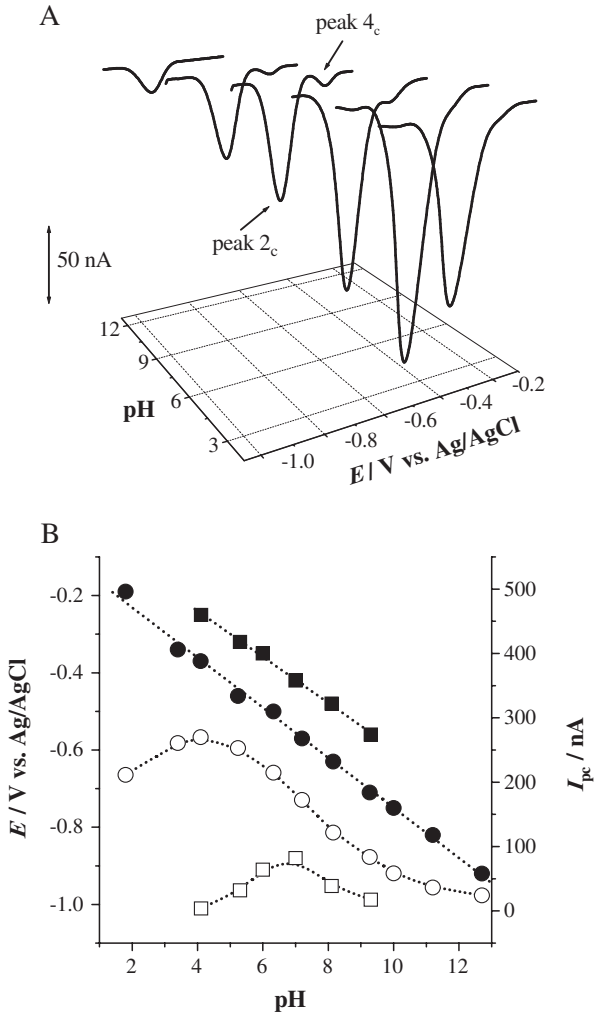

Fig. 6. A) 3D plot of DPV obtained in N_2 saturated solutions of 25 μM 2,8-DHA as a function of pH. B) Plots of (●) E_{pc} and (○) I_{pc} of peak 2_c and of (■) E_{pc} and (□) I_{pc} of peak 4_c vs. pH.

the solution of 2,8-DHA the electrode was washed with a jet of deionized water and then transferred to the supporting electrolyte. The voltammogram obtained in these conditions, Fig. 4B, shows the oxidation of 2,8-DHA, peak 1_a and of $P_{2,8\text{-DHA}}$, peak 3_a. On a second DP scan, peak 1_a disappeared due to a complete oxidation of adsorbed 2,8-DHA molecules. On the other hand, peak 3_a could still be identified with a higher current. In fact, consecutively recorded DP voltammograms in buffer showed only after the 3rd scan a continuous, but slow, decrease of the peak 3_a oxidation current.

3.2. Reduction

3.2.1. Cyclic voltammetry

The reduction of 2,8-DHA at a GCE was first studied in pH 4.5 0.2 M acetate buffer solutions saturated with N_2. Cyclic voltammograms at $v = 50$ mV s^{-1} recorded in 25 μM 2,8-DHA, Fig. 5A, revealed that the reduction of 2,8-DHA is a reversible process corresponding to the pair of peaks 2_c–2_a. Also, a small peak 4_c appeared as a shoulder preceding the pair of peaks 2_c–2_a.

The effect of molecular oxygen on the reduction of 2,8-DHA, was studied briefly by cyclic voltammetry in normal atmosphere for different scan rates, Fig. 5B. It was always possible to record the cathodic peak, but the corresponding anodic peak expected in the case of a reversible electrode process was observed only for scan rates higher than 100 mV s^{-1}. Hence, it is necessary to scan the potential faster than the reaction rate between O_2 and the reduced 2,8-DHA molecules such that, at the electrode surface there exist reduced 2,8-DHA molecules that could be oxidized when the scan direction is switched toward positive values.

On the other hand, the peak 2_c potential in the N_2 saturated solution is about 100 mV more positive than the potential value recorded in normal atmosphere. Also, in the presence of O_2, the reduction current is several times higher than in the N_2 saturated solution. Nevertheless, these two facts suggest that these peaks correspond to different electrode processes, the last one involving the participation of molecular oxygen.

3.2.2. pH effect; Differential pulse voltammetry

The pH study of 2,8-DHA reduction, peak 2_c, was performed in N_2 saturated solutions of 25 μM 2,8-DHA in a wide pH range from 1 to 13 using DPV, Fig. 6A.

For pH < 4.5 the DP voltammograms showed only one main reduction peak 2_c. However, for 4.5 < pH < 9.3, besides peak 2_c a new cathodic peak 4_c was observed at less negative potential values and always with a smaller current, Fig. 6A.

The potential of peak 2_c is displaced to more negative values with increasing pH. The dependence is linear over the whole pH range following the equation E_{pc} $(V) = -0.10 - 0.061$ pH, Fig. 6B. The slope of the line, 61 mV per pH unit, suggests that the reduction of 2,8-DHA involves the same number of electrons and protons. Taking into account the width at half-height of peak 2_c, $W_{1/2} = 44$ mV, it could be concluded that the reduction

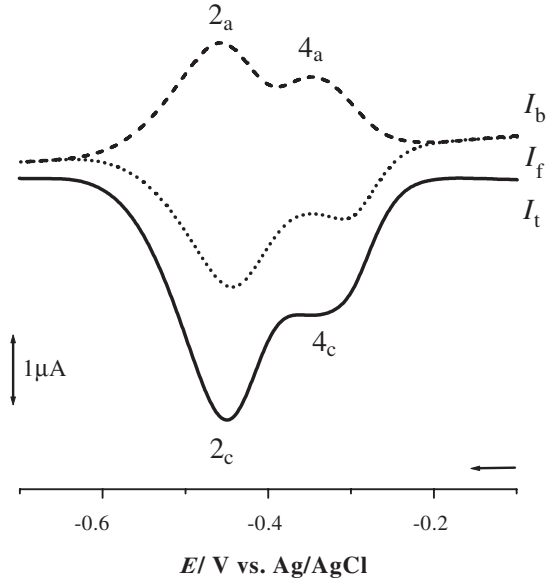

Fig. 7. SWV obtained in a N_2 saturated solution of 25 μM 2,8-DHA in pH 4.5 0.2 M acetate buffer: (—) I_t– total current, (·····) I_f– forward current and (- - -) I_b– backward current; $f = 50$ Hz, $\Delta E = 1$ mV, $v = 50$ mV s^{-1}.

of 2,8-DHA occurs with the transfer of 2 electrons and 2 protons.

The graph of the variation of peak 2_c current versus pH, Fig. 6B, shows that the current increases with the pH reaching a maximum in pH 4.5 0.2 M acetate buffer. For this reason, this electrolyte was chosen to study the reduction mechanism of 2,8-DHA. After this pH value, the current decreases with the pH increase.

Nevertheless, for pH between 4.5 and 9.3 the peak 4_c became more negative with increasing the pH of the supporting electrolyte, Fig. 6B. The peak potential turns more negative with 59 mV per pH unit and this variation showed that the number of electrons is equal with the number of protons transferred during this reduction. Also, it could be verified that the peak current shows a maximum in pH 7 0.2 M phosphate buffer, Fig. 6B.

3.2.3. Square wave voltammetry

SW voltammograms were also recorded in N_2 saturated solution of 25 μM 2,8-DHA in pH 4.5 0.2 M acetate buffer, using an effective scan rate $v = 50$ mV s^{-1} ($f = 50$ Hz, $\Delta E = 1$ mV), Fig. 7.

This SW voltammogram shows the reversible character of both charge transfer reduction reactions of 2,8-DHA. Moreover, the identical values of the peak potentials on the forward (I_f) and backward (I_b) currents shows that charge transfer is mostly due to the 2,8-DHA molecules adsorbed on the electrode surface [20].

4. Conclusion

This study shows that 2,8-dihydroxyadenine, the main in vivo adenine oxidation product, undergoes oxidation and reduction at a glassy carbon electrode.

The oxidation of 2,8-DHA is a pH dependent process and occurs in a single step, with the transfer of 2 electrons, leading to the formation of an electroactive oxidation product that strongly adsorbs on the GCE surface. Using cyclic voltammetry it was also possible to detect the formation of 2,8-DHA dimers.

The reduction of 2,8-DHA is a reversible process in the absence of molecular oxygen. In electrolytes with pH between 4 and 9 two consecutive reversible charge transfer reactions were identified. It was also demonstrated that O_2 interfered with the reductive electron transfer process of 2,8-DHA, and that in the presence of oxygen, the reduction of 2,8-DHA occurs at more negative potentials than in the absence of oxygen.

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