Electrochemical determination of 8-oxoguanine in the presence of uric acid

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Abstract

The electrochemical oxidation of 8-oxoguanine (8-oxoG) in the presence of uric acid (UA) was studied by differential pulse voltammetry over a wide pH range (1–12). The results showed that both compounds follow a pH-dependent oxidation mechanism that involves two electrons and two protons corresponding to reversible charge transfer reactions. The difference between the peak potential for the oxidation of each analyte was found to be always less than 100 mV over the whole pH range, the separation being greater in the pH interval 4–7. In mixtures of both analytes, pH 6 was shown to be the best for 8-oxoG determination in the presence of uric acid, since the peak current is higher and a greater peak separation is achieved.
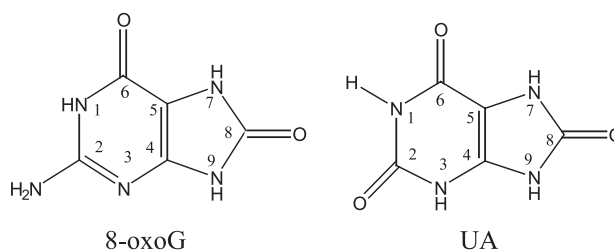© 2004 Elsevier B.V. All rights reserved.

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1. Introduction

DNA is under constant attack from free radical species leading to the occurrence of multiple modifications, including base-free sites and oxidised bases that are potentially mutagenic [1]. Guanine is the most easily oxidised base in DNA and the major product of guanine oxidation is 8-oxoguanine (8-oxoG), which is widely accepted as a biomarker of DNA oxidative damage [2]. Elevated levels of 8-oxoG were found in urine of smokers and patients with several diseases including cancer, immunodeficiency syndrome and neurodegnerative diseases [3]. Endogenous production of 8-oxoG due to aerobic cellular metabolism leads to basal levels of one 8-oxoG/10^6 DNA bases, being excreted into urine at basal concentrations below 100 nM [4]. The assessment of 8-oxoG in urine provides a non-invasive approach to evaluate the extent of DNA oxidative damage and DNA repair. Due to their high sensitivity, voltammetric techni-ques enable the detection of such low concentrations of 8-oxoG [5].

8-oxoG   UA

In urine, purines such as uric acid (UA) are co-excreted in concentrations 10^4-fold higher than 8-oxoG. UA is the major end product of purine metabolism in humans and its levels in urine are also indicative of various diseases. Uric acid has a structure very similar to 8-oxoG and is thus a strong interferent.

In this work, the dependence of the oxidation peak potential of both substances with pH over the pH range 1–12 was studied by differential pulse voltammetry in order to optimise the experimental conditions and pH interval for good voltammetric separation of these compounds.

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2. Experimental

Solutions of 5 μM 8-oxoG, 5 μM UA and equimolar 5 μM mixtures of 8-oxoG and UA were prepared by direct dilution of the appropriate volume from stock solutions in sodium acetate, phosphate or borate buffer according to the pH desired. In all cases buffer electrolyte solutions of ionic strength 0.1 were used and were prepared using analytical grade reagents (Sigma-Aldrich, Madrid) and purified water from a Millipore Milli-Q system (conductivity <0.1 μS cm^{-1}).

All voltammetric experiments were performed using a μAutolab running with GPES version 4.9 software, Eco-Chemie, Utrecht, The Netherlands. A glassy carbon electrode (GCE) ($d=6$ mm) as working electrode, a Pt wire as counter electrode and a saturated calomel electrode (SCE) as reference electrode, were used in a 10 ml one compartment electrochemical cell. The differential pulse voltammetry [6] experimental conditions were:, pulse amplitude 50 mV, pulse width 70 ms, scan rate 5 mV s^{-1}. All potentials are referred to SCE.

3. Results and discussion

The differential pulse voltammograms of a 5 μM solution of 8-oxoG, UA and a 5 μM equimolar mixture of 8-oxoG and UA in pH 6.2, 0.1 M phosphate buffer and in pH 9.4, 0.1 M sodium borate buffer, respectively, are presented in Fig. 1A and B.

The oxidation of 8-oxoG occurs in pH 6.2 buffer at $+0.36$ V and in pH 9.4 buffer at $+0.13$ V. The peak width

at half height ($W_{1/2}$) is approximately equal to 58 mV at both pHs indicating that two electrons are transferred in the oxidation of 8-oxoG, in good agreement with a previous report [5]. UA oxidation occurs in pH 6.2 buffer at $+0.29$ V and in pH 9.4 buffer at $+0.09$ V. Also $W_{1/2}$ is approximate 58 mV indicating the transfer of two electrons in the UA oxidation. However, at all pHs and for the same concentration, 8-oxoG always gives a peak with higher current than UA.

In an equimolar mixture of 8-oxoG and UA at pH 6.2, the oxidation peak potential values are separated by less than 100 mV (Fig. 1A), which compromises their simultaneous detection. The mixture at pH 9.4 (Fig. 1B) presents only one peak with a potential close to that of 8-oxoG, and it is not possible to separate the peak from UA. The separation between the oxidation peaks is very reproducible over the whole pH range and a maximum peak separation of 60 mV is achieved in the pH range 4–7, as can be seen in Fig. 1A.

It is shown that, in the mixture, the 8-oxoG peak current decreases substantially compared to that obtained when UA is not present. This indicates a competition effect between both compounds for the active electrode surface.

For a better understanding of the real interference of UA in the electrochemical determination of 8-oxoG, the dependence of peak potential and peak current for oxidation of both substances with pH was studied over the range pH 1–12. The purpose was to find at which pH values the peak separation for these very similar electroactive compounds is maximum and the peak current for UA is comparatively lower in order to minimise its interference. The results obtained are presented in Fig. 2
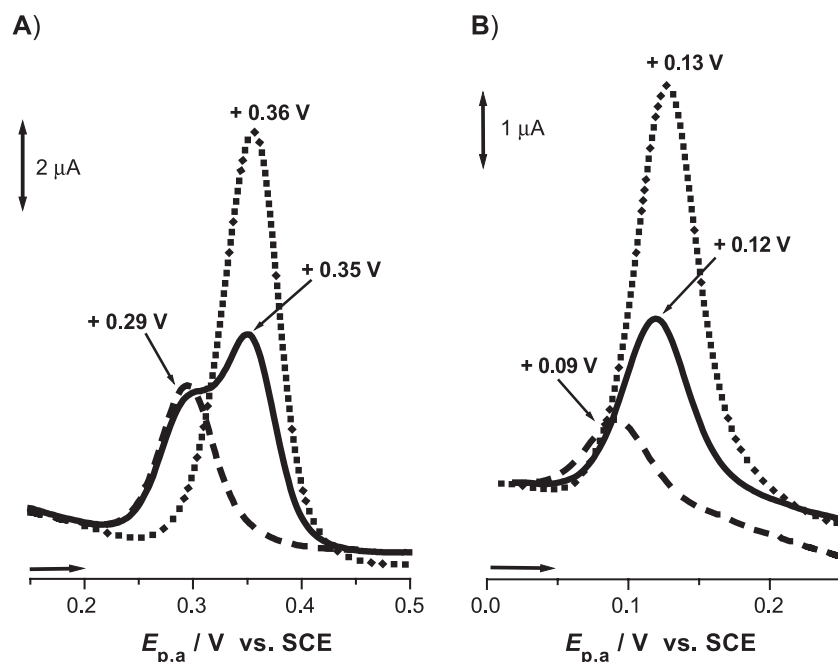

Fig. 1. Differential pulse voltammograms of 5 μM solution of (····) 8-oxoG, (– – –) UA and (——) 5 μM equimolar mixture of 8-oxoG and UA in: (A) pH 6.2, 0.1 M phosphate buffer and (B) pH 9.4, 0.1 M sodium borate buffer. Pulse amplitude 50 mV, pulse width 70 ms and scan rate 5 mV s^{-1}.
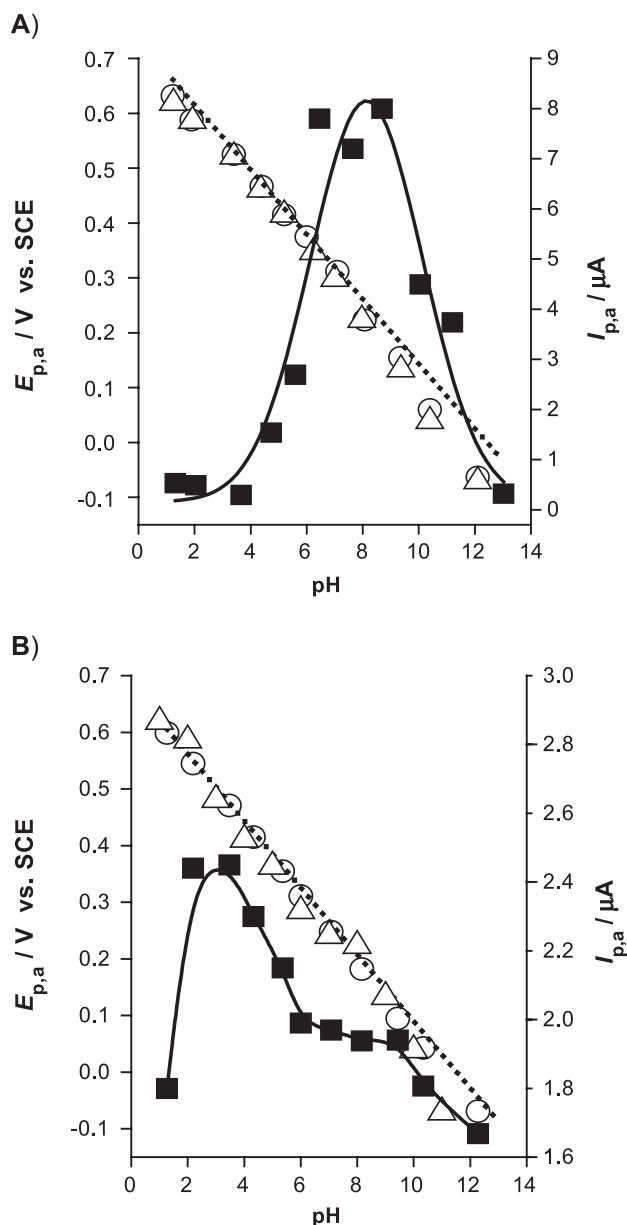
Fig. 2. Plot of E_{pa} (◯) and I_{pa} (¦) vs. pH for 5-μM solutions of (A) 8-oxoG and (B) UA, together with plots of E_{pa} (△) vs. pH for 5 μM equimolar mixture of both compounds. Dotted lines slope 59 mV per pH unit.

and show that peak current for 8-oxoG is maximum in the pH range 6–8 (Fig. 2A), while for UA, higher peak currents are obtained at pH lower than 5 (Fig. 2B). For both analytes, the peak potential follows a linear dependence with pH with a slope of 59 mV/pH (Fig. 2A and B). The pH and nature of the supporting electrolyte influence the peak potentials. With both acetate and phosphate buffers, in the pH interval of 4–7, the peak separation is a maximum. Nevertheless, for both substances the variation of peak potential with pH over the whole range studied still fits to a line of slope 59 mV/pH, meaning that the same number of electrons and protons are involved.

Over the entire pH range studied, the observed $W_{1/2}$ for each analyte is close to 60 mV, which, together with the pH dependent oxidation peak potential, leads to the conclusion that both compounds follow a reversible charge transfer mechanism that involves two electrons and two protons.

The behaviour of equimolar mixtures of both analytes was studied, and it was observed that the presence of UA does not interfere significantly with the peak potential for 8-oxoG oxidation. Nevertheless, a small shift to a lower potential of the 8-oxoG peak when UA is present was observed. This is shown in Fig. 2A, a plot of the 8-oxoG peak potential pH dependence in the absence or presence of UA, and in Fig. 2B, is presented the UA peak potential pH dependence in the absence or presence of 8-oxoG. It is confirmed that it is only possible to distinguish the 8-oxoG oxidation peak from the UA oxidation peak in the pH range 4–7, since at all other pHs only one peak could be observed, as shown in Fig. 1.

In mixtures of both compounds, the 8-oxoG peak current decreases substantially when compared with the peak current obtained in the absence of UA. A pH value of 6 is best for 8-oxoG determination since the peak current is bigger and sufficiently good separation between peaks is obtained allowing the electrochemical determination of 8-oxoG in the presence of uric acid to be achieved. It was possible using the optimised experimental conditions to obtain a detection limit of 10 fmol for 8-oxoG in the presence of UA in standard mixtures [7].

4. Conclusions

8-oxoG has very similar voltammetric behaviour to uric acid. Both compounds follow a pH-dependent oxidation mechanism that involves two electrons and two protons corresponding to reversible charge transfer. The pH ranges for the maximum current and peak separation leads to the conclusion that the pH range 6–8 should be used for 8-oxoG detection in the presence of UA. In mixtures of both compounds, the 8-oxoG peak current decreases substantially when compared with the peak current obtained in the absence of UA and pH 6 showed to be the best for 8-oxoG determination since the peak current is higher and good separation between peaks is achieved.

Acknowledgements

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