Electrochemical Oxidation of 8-Oxoguanine

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Abstract

Electrochemical oxidation of DNA can occur at each of the four bases and guanine is the one that can suffer the easiest oxidative damage. The occurrence of the guanine oxidation product, 8-oxoguanine, as a consequence of DNA damage caused by DNA oxidation causes important mutagenic lesions and hence it is very important to develop reliable methods for its quantification. Electrochemical study of the mechanism of oxidation of 8-oxoguanine on glassy carbon shows that it is a reversible electrode process, pH dependent, and involves several reaction products. Electroanalytical determinations of 8-oxoguanine were carried out and the detection limit was $8 \times 10^{-7}$ M.

Keywords: 8-Oxoguanine, Electroanalysis, Electrochemical oxidation, Oxidative stress, DNA oxidation

Dedicated to Professor Emil Paleček on the Occasion of His 70th Birthday

1. Introduction

The in vivo oxidation of chromosomal and mitochondrial DNA causes cell damage and plays an important and probably the central role in mutagenesis, carcinogenesis, autoimmune inflammatory diseases, neurodegenerative diseases, atherosclerosis, ischemia/reperfusion injury, stroke and chronic respiratory disease, and has been suggested to be a major contributor to aging and other age-related diseases [1–10].

As a consequence of the oxidative lesion of DNA [2, 11, 12], the compound commonly referred to as 8-oxoguanine (7,8-dihydro-8-oxoguanine or 8-oxoG) has been identified as the product of oxidation of guanine in the C8 position [8, 13–17] and the structure is shown in Scheme 1.

Scheme 1

The 8-oxodeoxyguanosine (8-oxodG), molecule exists predominantly in the 6,8-diketo tautomeric form in solution and therefore its correct name is 7,8-dihydro-8-oxo-2′-deoxyguanosine [13].

8-Oxoguanine can arise from DNA through direct attack of reactive oxygen species on chromatin, or from oxidation damage of the nucleotide pool, namely, from 8-oxo-7,8-dihydro-2′-deoxyguanosine-5′-triphosphate during DNA synthesis [18]. It is directly associated with promutagenic events and other cellular disorders both in vivo and in vitro [2].

The formation of 8-oxoguanine in the DNA moiety, considered the most commonly measured product of DNA oxidation [2, 8], causes important mutagenic lesions [19]. In the DNA double helix this adduct pairs more easily with adenine (A) than with cytosine (C) [4, 5, 20]. This could lead to the substitution of cytosine in the complementary chain by adenine, which in turn leads to the substitution of the original guanine (G) by thymine (T). Through this consecutive series of events, an oxidative injury to DNA could result by a mutagenic transversion of the type G → T [1]. A mutation of G:C to T:A could then occur and be the starting point for a cellular dysfunction (malfunction), which in turn could lead to a state of illness [2–5]. This transversion mutation is also often observed spontaneously in many tumor cells [21].

In cancer tissues [2, 20, 22], as well as in lung tissues of smokers [19, 23], the levels of 8-oxoguanine found are higher than in healthy tissues, where a steady-state level exists due to the normal products of metabolism that oxidize DNA [2, 8]. Higher levels have also been found in laboratory tests animals with chronic infectious diseases or under great DNA oxidative stress [8–10, 19, 22–28]. Some controversial studies show that 8-oxoguanine accumulates with age in mitochondrial DNA [26].

However, since 8-oxoguanine from the diet is not assimilated by the organism all the secreted 8-oxoguanine detected, usually in urine, is a direct consequence of DNA oxidation [19]. Consequently this compound has been proposed as a urine biomarker for DNA oxidative lesions [24, 25], establishing a key step in the effort to link oxidant formation to biomolecular damage and disease initiation and progression in a causative fashion [27].

Initially, high performance liquid chromatography with electrochemical detection (HPLC-ECD) was used to quantify 8-oxoguanine and 7,8-dihydro-8-oxo-2′-deoxyguanosine in DNA hydrolysates as a measure of the steady-state level of DNA oxidation in situ [28].

Since then, different methods based on HPLC-ECD, gas chromatography-mass spectrometry (GC-MS), immunoaffinity columns with specific antibody, avidin and analogues, and enzymatic methods of extraction and digestion of the samples, have been developed and improved for the analytical determination of 8-oxoguanine [2, 8–10]. However, the values found by the different methods for the same source of sample give very different results over several orders of magnitude. In general, the levels found for 8-oxoguanine with HPLC are lower than those found by GC-MS, and higher than those determined by other methods [2, 8, 10].

In this work an electrochemical study of the mechanism of oxidation of 8-oxoguanine on glassy carbon is presented in order to develop an electroanalytical procedure that could be applied to future determinations in biological fluids.
2. Experimental

Guanine from Sigma Chemical Co. and 8-oxoguanine from Aldrich (2-Amino-6,8-dihydroxypurine) were used without further purification.

Solutions of 0.10 mM 8-oxoguanine were prepared in acetate, phosphate, or borate buffer, plus potassium chloride or hydroxide solutions according to the pH desired. Solutions of ionic strength 0.1 were used in all experiments and were prepared using analytical grade reagents and purified water from a Millipore Milli-Q system (conductivity < 0.1 \mu S cm^{-1}). The pH measurements were carried out with a Crison 2001 pH-meter with a combined glass electrode. All experiments were done at room temperature.

Differential pulse voltamograms were recorded using an Autolab PGSTAT 10 running with GPES version 4.3, software PG (Eco-Chemie, Utrecht, The Netherlands). The differential pulse voltammetry conditions used were: pulse amplitude 50 mV, pulse width 70 ms, and scan rate 5 mV s^{-1}. The working electrode was glassy carbon with a surface area of 0.9 cm^{2}, the counter electrode was a Pt wire, and the reference electrode was Ag/AgCl (sat KCl), all contained in a one-compartment electrochemical cell, with a volumetric capacity of 10 mL. Some differential pulse voltammograms were recorded after conditioning the working electrode in the 8-oxoguanine solution at +1.4 V during 180 s. Cyclic voltammetry scan rates were 10, 50, 100, and 200 mV s^{-1}.

3. Results and Discussion

The oxidation product of guanine, 8-oxoguanine, in aqueous solution presents tautomeric forms of which, according to NMR studies [17], the 6,8-diketo (I) is predominant (see Scheme 2).

![Scheme 2](image)

The electrochemical oxidation mechanism of 8-oxoguanine is a multistep process in which a main peak is observed corresponding to the oxidation of 8-oxoguanine followed by other small peaks due to 8-oxoguanine oxidation products. Differential pulse voltammetry of 8-oxoguanine showed one very well-defined anodic peak at pH 3.6 at a potential of \( E_{p,a} = +0.580 \text{ V (vs. Ag/AgCl) } \), and a width at half height \( W_{1/2} = 49 \text{ mV} \), Fig. 1. However, as can be seen in the figure the shoulders suggest that there are other electrode processes involved as well. In the literature [29], it is described that oxidation of 8-oxoguanine by reactive oxygen species (depending on reaction conditions) generates a complex mixture of products which has not yet been fully characterized. Although practically no adsorption was found for oxidation of 8-oxoguanine at pH < 9.0, both 8-oxoguanine or the oxidation products adsorb strongly on the electrode surface for pH > 9.0. One of the products could be 2,6-diamino-4-hydroxy-5-formamidopyrimidine (FAPyG) (Scheme 3), corresponding to breaking of the N7–C8 bond of 8-oxoguanine [2]. Cyclic voltammetry of 8-oxoguanine, (Fig. 2) showed that oxidation at a glassy carbon electrode is diffusion controlled,

![Fig. 1](image)

![Fig. 2](image)
but due to very fast hydrolysis [30, 31] the reverse peak is not well defined. However, square-wave voltammetry (Fig. 3) using an effective scan rate of 100 mV s\(^{-1}\), shows the existence of a reverse peak corresponding to reduction of the adsorbed oxidation product.

Scheme 3

The electrochemical oxidation of 8-oxoguanine was also studied using differential pulse voltammetry for a wide range of pH (1.1 to 13.1), and it followed a two-electron two-proton transfer pathway over all the pH range studied (Fig. 4a). Peak currents increased with increasing pH and the peaks almost disappeared for pH values lower than 2 (Fig. 4b).

The results showed that 8-oxoguanine is more easily oxidized than guanine, which has a much higher oxidation potential for the same experimental conditions [30–33] (Fig. 5). A differential pulse voltammogram obtained for a solution mixture of 0.10 mM 8-oxoguanine and 0.09 mM guanine, in pH 4.5 0.1 M acetate buffer, shows two very well separated anodic peaks. The first peak corresponds to the oxidation of 8-oxoguanine at \(E_{p,a} = +0.513\ V\), \(W_{1/2} = 49\ mV\) and the second to the oxidation of guanine at \(E_{p,a} = +0.825\ V\), \(W_{1/2} = 54\ mV\), and the oxidation of both 8-oxoguanine and guanine involve two electrons and two protons. This demonstrates that it is possible to easily distinguish these two compounds, if both are present in the same sample, using differential pulse voltammetry. In fact, selective amperometric detection of these two compounds employing HPLC-ECD has been described [17, 29].

Since 8-oxoguanine at pH < 9.0 does not cause poisoning of the electrode the possibility of preconcentration of 8-oxoguanine on the electrode surface by applying a potential of +1.4 V for
standards a straight line calibration plot was obtained described experiments. From the differential pulse voltammograms of the scan. It was not necessary to clean the electrode between without applying any conditioning potential before the potential

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180 s before the potential scan was investigated, as shown in Figure 6. In this way there was an increase on the peak height so that with preconcentration lower detection limits can be attained

Electroanalytical quantification of 8-oxoguanine was done in pH 4.5 0.1 M acetate buffer. Pulse amplitude 50 mV, pulse width 70 ms and scan rate 5 mV s\(^{-1}\), electrode held at 0 V for 5 s between each potential scan. (-----) without any conditioning potential applied; (———) with a conditioning potential of +1.4 V applied for 180 s before the scan.

by the equation \( I(\mu A) = 0.43 \) [8-oxoguanine/\( \mu M \)] + 0.19 \((r = 0.989, n = 5, SD = 0.719)\), for 8-oxoguanine and the detection limit was \( 8 \times 10^{-7} \) M, based on three times the noise level.

4. Conclusions

The electrochemical oxidation of 8-oxoguanine is a reversible electrode process. It is pH dependent and involves several reaction products. Electroanalytical determinations can be done and the detection limit found was \( 8 \times 10^{-7} \) M.

This electroanalytical method augurs well for the determination of 8-oxoguanine in biological fluids, such as serum or urine, of patients suffering from oxidative stress and which can be the cause of other disorders such as cancer or age related diseases.

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6. References

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