Sonoelectrochemical studies of guanine and guanosine
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Abstract

The voltammetric behaviour of the purine base guanine and the corresponding nucleoside guanosine at a glassy carbon electrode was investigated with the help of ultrasound. The adsorption of guanine and guanosine as well as the adsorption of their oxidation products affects the overall voltammetric characteristics dramatically. In particular, the effect of ultrasound on the simultaneous adsorption of guanine and guanosine was studied in detail. Quantitative sonovoltammetric experiments show the number of electrons involved in the electro-oxidation of guanosine to be two. Sonovoltammetry, i.e. the combination of voltammetry and ultrasonic irradiation, is demonstrated to be a useful approach to control the extent of adsorption of the relevant species and to avoid electrode fouling. This allows the development of reliable analytical procedures for the determination of guanine and guanosine, which are described in detail. © 1997 Elsevier Science S.A.

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1. Introduction

Purine derivatives have great significance in various biological processes. For example, the nucleotides of adenine and guanine can be regarded as monomer units of the nucleic acids. In order to gain deeper understanding of complex biological systems it is important to investigate the building blocks in detail.

The electrochemical behaviour of purine derivatives has been addressed in several previous reports. It was found that many of them can be reduced at mercury electrodes [1-3] and oxidized at carbon-based electrodes [4,5]. The present paper deals specifically with the voltammetric properties of guanine and the corresponding nucleoside guanosine which have the following chemical structures:

![Guanine and Guanosine](image)

The electrochemical oxidation of guanine has been reported to proceed as a two-step mechanism with 8-oxyguanine as intermediate and involves the total loss of four electrons and four protons [6]. However, not much is known about the reaction mechanism of the electrochemical oxidation of guanosine [7]. Regarding the electroanalytical determination of guanine and guanosine, there is still some controversy in the literature as to whether or not adsorption plays a role in the analytical procedure. Dryhurst found that adsorption has an enormous influence on the determination of guanine and guanosine when using a pyrolytic graphite electrode [7]. Similar behaviour was found for glassy carbon electrodes [8]. In contrast to this, Gilmartin and Hart [9] reported guanine determinations at carbon paste and glassy carbon electrodes where no effect of adsorption was observed.

The present work is concerned with mechanistic and analytical investigations of guanine and guanosine with the help of ultrasonic irradiation. Ultrasound-assisted electrochemistry is attracting growing interest of various research groups [10-13]. In addition to fundamental studies [14], some analytical applications have also been reported [15]. In a recent paper [16] we described a small-volume sonovoltammetric cell which is well suited for analytical sonovoltammetry. From the analytical point of view there are two important main effects of ultrasonic irradiation on voltammetric experiments. Firstly, the mass transport is dramatically enhanced and secondly the electrode surface can be activated in situ, acting as a strategy against electrode fouling. Both effects were utilized in the present
study to investigate the voltammetric characteristics of guanine and guanosine and to develop reliable procedures for their determination.

2. Experimental

2.1. Apparatus and equipment

The cell configuration used for the sonovoltammetric experiments is illustrated in Fig. 1. The jacketed cell was thermostatted by circulating water from a constant temperature bath (25 °C). The volume of the cell electrolyte was always 20 ml. A platinum coil was used as counter electrode and a laboratory-made silver/silver chloride/3 M KCl electrode served as reference electrode. The glassy carbon working electrode (d = 6 mm) was positioned so as to face the tip of the sonnic horn. The horn was connected with a tapered microtip (d = 3 mm) which was fabricated from high grade titanium alloy. The ultrasonic processor was a model VC501 (Sonics and Materials Inc., USA) capable of delivering up to 500 W at 20 kHz frequency. The ultrasonic processor is designed to deliver constant amplitude which can be selected via the amplitude control setting ranging from '0' to '100'; however, in conjunction with the microtip the amplitude control setting must not be higher than '40'. The actual power intensity entering the system was calibrated calorimetrically according to the procedure of Mason et al. [17]. For relevant amplitude control settings of '10', '15' and '20' the corresponding power intensities were 16 ± 3 W cm⁻², 30 ± 3 W cm⁻² and 72 ± 5 W cm⁻² respectively. The sonovoltammetric cell and the sonic horn were housed in a sound proofed cage in order to protect the operator from high-intensity acoustic noise.

All voltammetric experiments were done using an Autolab PGSTAT10 potentiostat (Eco Chemie, Utrecht, Netherlands) equipped with an ECD low current module. The current signal was filtered through a third-order Sallen-Key filter with a time constant of 0.1 s in order to remove high frequency i.c. components.

The glassy carbon electrode (GCE) was prepared for measurement by polishing using plastic foils (Hirschmann, Germany) with adherent alumina of decreasing particle size ranging from 9 µm to 0.3 µm, followed by thorough rinsing with Milli-Q water. Prior to recording voltammograms of electroactive species, several cyclic voltammograms were recorded in the background solution until a stable voltammetric response was obtained.

2.2. Chemicals and solutions

Guanine and guanosine were obtained from Sigma Chemical Co. and were used as received. The complex K₄[W(CN)₆]. 2H₂O was prepared according to the literature [18]. All solutions were made up using high-purity water from a Millipore Milli-Q system (resistivity greater than or equal to 18 MΩ cm) and analytical reagent grade chemicals.

An acetate buffer containing 0.1 M sodium acetate + acetic acid with a pH of 4.50 and a 0.1 M phosphate buffer (pH 7.00) served as supporting electrolytes. Solutions of the purine derivatives were prepared directly in the buffer solutions except in the case of guanine. Stock solutions of guanine of 1 mM were made either in 0.1 M NaOH or in 0.1 M HClO₄. Working solutions of guanine were prepared by adding small volumes of stock solution to the acetate buffer solutions. The solutions were then sonicated to ensure homogenization.

3. Results and discussion

3.1. Voltammetric and sonovoltammetric characterization of guanine

One problem of studying the electrochemical behaviour of guanine is its low solubility at pH values where it is a neutral molecule. Dryhurst [7] reported a concentration of about 5 × 10⁻⁴ M for saturated guanine solutions in the pH range between 4 and 7. As will be specified later, our results indicate an even lower concentration for saturated solutions of guanine in acetate buffer. However, owing to the protolytic properties of guanine, in acids and bases it is possible to dissolve appreciable amounts of guanine because it is transformed into an ionic form (pK₁ = 3.0, pK₂ = 9.3, pK₃ = 12.6 [19]). This was utilized for preparing 1 mM guanine stock solutions of accurately known concentration.

The adsorption properties of guanine can be deduced from cyclic voltammograms recorded in the presence and
absence of ultrasound. In the absence of ultrasound the current response decreases progressively when recording successive voltammograms (see Fig. 2 traces 1a and 1b). This is probably for two reasons. There is adsorptive accumulation of guanine at the electrode surface which leads to a higher signal in the first scan, and the products of oxidation remain partially adsorbed resulting in some surface blockage. The adsorption of guanine was further studied through transfer experiments where the GCE was left for a certain time at open circuit potential in an acetate buffer solution containing $2 \times 10^{-5}$ M guanine and was then transferred to a pure buffer solution after careful cleaning with a jet of deionized water. In the first scan a signal corresponding to guanine was obtained, the size of which was clearly dependent on the accumulation time. This behaviour was qualitatively the same either in pH 4.5 acetate or pH 7.4 phosphate buffer solution.

In the presence of ultrasound, the voltammetric response was independent of previously recorded scans as illustrated in Fig. 2, traces 2a and 2b. Reproducible measurements could be taken without the effects of electrode fouling. However, it seems that, even with sonication, adsorption has some effect on the voltammetric response as demonstrated by cyclic sonovoltammograms of guanine which have the peak-shaped response of the forward scan illustrated in the cyclic sonovoltammogram shown in Fig. 3. These show a slight difference between the half-wave potentials of the forward and reverse scans respectively. Under these conditions the wave height of the reverse scan was used for quantitative determinations because in that case pure mass transport control can be assumed.

The surface state of the glassy carbon electrode resulting from potential scans between 0.1 V and 1.4 V promotes guanine adsorption in comparison with the potential range scanned for the results in Fig. 2 (0.5-1.1 V). It should be added that various experiments without sonication were conducted in order to find alternative possibilities for activating the electrode surface between successive measurements. These attempts included the application of several conditioning potentials prior to recording single sweep, differential pulse or square wave voltammograms. However, no procedure gave results as good as those obtained by performing sonovoltammetric measurements.

3.2. Voltammetric and sonovoltammetric characterization of guanosine

The solubility of guanosine is much better than that of guanine which allows studies at concentrations up to the millimole range. Fig. 4 shows successive cyclic voltammograms of guanosine in the absence of ultrasound. Similar to the behaviour obtained for guanine, there is a progressive decrease in the current response for repetitive scans. In contrast to this, repetitive cyclic sonovoltammograms show no signal degradation as illustrated in Fig. 5.
measurements at various concentrations of guanosine it was found that only the main signal at 1.15 V depends clearly on the bulk concentration of guanosine.

The most probable explanation for the above results is that the guanosine used contained some guanine impurity (less than 1%). Such an observation was also reported by Dryhurst [7] for many commercial samples. Indeed, in Fig. 5, the small wave (0.75-0.95 V) that appears in the cyclic sonovoltammograms of 0.1 mM guanosine solutions can be attributed to the oxidation of guanine.

The adsorption of guanosine was studied in solutions containing guanosine at very low concentration, where signal contributions related to mass transport from the bulk solution during the recording can be neglected. After an accumulation period of 10 min at 0 V in a solution containing $2.5 \times 10^{-6}$ M guanosine, the first cyclic voltammogram shows two signals at 0.88 V and 1.1 V, Fig. 6(A,a). The cyclic voltammograms shown in Fig. 6(A,b) were recorded under identical conditions except that immediately before starting the recording a potential of 0.95 V was applied for 10 s. This procedure results in selective removal of adsorbed guanine which gives an oxidation signal at 0.88 V, while the second signal is not affected.

In addition, Fig. 6(B) shows that the accumulation of guanosine in the presence of ultrasound results in higher signals at 0.88 V than at 1.1 V in comparison with accumulation in silent solution. This suggests that the species oxidized at 0.88 V, guanine, is the more strongly adsorbed, since the other, guanosine, can be partially removed by ultrasonic irradiation. Concerning the very small amount of guanine impurity, the high sensitivity of the adsorptive sonovoltammogram for the guanine adsorbed on the electrode surface is remarkable — its concentration can be estimated to be in the nanomolar range.

Fig. 7 shows the response obtained when the electrode was immersed in 0.1 mM guanosine solution for 5 min and then transferred to a pure buffer solution for recording the cyclic voltammograms. The second peak of guanosine at 1.1 V is substantially larger than that of guanine at 0.88 V. This shows that the second signal is not due to further oxidation of products formed in the first oxidation step at 0.88 V, because it corresponds to a much higher concentration of guanosine adsorbed on the electrode surface than the small peak from oxidation of adsorbed guanine.

Experiments were undertaken in order to determine the number of electrons transferred during oxidation of guano-
sine. For this a sonovoltammogram of a mixture of K₄[W(CN)₆] and guanosine was recorded, Fig. 8. The limiting current $I_{lim}$ derived from a sonovoltammogram can be described by the following equation

$$I_{lim} = knFADc$$

where $k$ is an empirical coefficient related to the experimental parameters, $n$ is the number of electrons transferred, $F$ is the Faraday constant, $A$ is the electrode area, $D$ is the diffusion coefficient of the electroactive species and $c$ its bulk concentration. The octacyanotungstate complex shows a simple one-electron oxidation and is used as an internal standard in order to eliminate the empirical coefficient. The number of electrons transferred during guanosine oxidation multiplied by the ratio of the diffusion coefficients of guanosine and octacyanotungstate amounts to 2.1 ± 0.3. In a similar experiment performed with guanine instead of guanosine, a value of 4.8 ± 0.3 was obtained. Assuming that the diffusion coefficients of guanine and guanosine are similar, it can be concluded that the number of electrons transferred in the oxidation of guanosine is half that transferred for guanine. Guanine has been reported to be oxidized in a two-electron step, forming 8-oxyguanine which can be further oxidized in a second two-electron step resulting in a quinonoid-diimine species [6]. According to the structure of guanosine, an analogous oxidative electrode reaction leading to 8-oxyguanosine would be possible; however, further oxidation leading to a diimine species is not possible. Thus, a two-electron process can be assumed for the electro-oxidation of guanosine.

3.3. Analytical determinations of guanine and guanosine

Cyclic sonovoltammograms allow reliable determinations of the guanine concentration based on the measurement of the wave height of the reverse scan. The horn tip–electrode separation was 8 mm and a power intensity of 30 W cm⁻² was chosen. A linear dependence on concentration of the limiting current was obtained for guanine concentrations between $4 \times 10^{-7}$ M and $2 \times 10^{-3}$ M. The results of linear regression of the calibration data are

$$I_{lim} [\mu A] = 0.798 [\mu A/\mu M] \times c [\mu M] + 0.766 [\mu A]$$

with $n = 7$, and regression coefficient 0.9993.

Under these experimental conditions a detection limit of $2 \times 10^{-7}$ M guanine was determined based on a signal-to-noise ratio of 3 which compares favourably with previous d.c. voltammetric determinations at pyrolytic graphite electrodes (e.g. the concentration range studied in [7] is $4 \times 10^{-5}$ M to $5 \times 10^{-4}$ M). The remarkably low limit of detection obtained for linear sweep or cyclic voltammetric measurements reflects the high mass transport efficiency due to ultrasonic irradiation. Comparable low limits of detection for guanine have only been reported recently [9] based on differential pulse voltammetry using carbon paste electrodes ($1 \times 10^{-7}$ M) and glassy carbon electrodes ($7.5 \times 10^{-7}$ M).

Using the analytical procedure described above, concentrations in saturated guanine solutions were determined. Saturated guanine solutions were prepared by sonication of an acetate buffer containing excess solid guanine for 15 min and subsequent filtration. Appropriately diluted samples were measured using the method of multiple standard additions. The guanine concentration in a freshly prepared saturated solution was $2 \times 10^{-3}$ M. Somewhat higher concentrated guanine solutions ($3-4 \times 10^{-5}$ M) can be prepared by adding small volumes of the alkaline or acid stock solutions of guanine to an acetate buffer. However, the guanine concentration in these solutions tends to decrease progressively accompanied by sedimentation of guanine. According to our results the solubility of guanine in its neutral form is approximately an order of magnitude lower than reported previously [7,20]. However, an older report by Albert and Brown [21], in which the mass ratio between water and the soluble amount of guanine was determined to be 200 000 corresponding to a concentration of about $3 \times 10^{-5}$ M, confirms our result.

Guanosine was determined in the same way as described for guanine. However, background subtraction is necessary because the sonovoltammetric wave is close to the positive potential limit of the acetate buffer system.

Differential pulse voltammetry was used for guanine and guanosine determinations as an alternative to cyclic voltammetry. Ultrasound was applied only during the initial part of the voltammogram until a chosen potential of 0.65 V, in order to control the extent of adsorption during the determination. The final part of the voltammogram was recorded in silent solution because this leads to more precise signals than in the presence of ultrasound.

Fig. 9(A) illustrates the typical response obtained when recording differential pulse voltammograms of guanosine with ultrasonic irradiation during the initial part of the recording. Again the smaller peak appearing at 0.8 V...
corresponds to adsorbed species of guanine traces and was of constant size independent of varying guanosine concentrations (10\(^{-3}\)–10\(^{-6}\) M), whereas the signal at 1.05 V can be used to evaluate the concentration of guanosine.

Both guanine and guanosine can be reliably determined by this method. Linear calibration plots were obtained for both compounds. The detection limits were 8 × 10\(^{-7}\) M and 3 × 10\(^{-6}\) M for guanine and guanosine respectively. However, one must have in mind that during differential pulse experiments the ultrasound is switched off before the peaks. Consequently, the limit of detection for guanine is higher using differential pulse voltammetry instead of cyclic sonovoltammetry (2 × 10\(^{-7}\) M) and this method should therefore be preferred for determination of guanine at very low concentrations.

The voltammetric response was also studied for mixtures of guanine and guanosine. Fig. 9(B) shows differential pulse voltammograms for various guanosine concentrations in the presence of a constant concentration of guanine of 1.4 × 10\(^{-5}\) M. In the presence of guanosine of 10\(^{-5}\) M concentration or higher the guanine signal decreases by about 8% compared with a pure guanine solution. The reason is probably that guanosine adsorption displaces some adsorbed guanine which leads to a reduction in the guanine signal due to the reduced electrode area available to the reaction. However, both components could easily be determined in mixed solutions and gave linear calibration plots. The procedure described was found to be a reliable approach to determine guanine and guanosine as single compounds or in a mixture.

4. Conclusions

The combination of sonochemical and electrochemical techniques permits a detailed study of the adsorption behaviour of guanine and guanosine at glassy carbon electrodes. Owing to the high mass transport efficiency in the presence of ultrasound, even traces of guanine lead to easily detectable oxidation signals of adsorbed guanine which was shown to adsorb more strongly than guanosine. Sonovoltammetric determinations of guanine and guanosine, either separately or in a mixture, are characterized by high sensitivity and good reproducibility even for extended measuring periods. The latter criterion is a particular advantage over conventional voltammetric determination of these compounds and results from a continuous in situ activation of the electrode surface and a well defined control of adsorption of the analytes by ultrasonic irradiation.

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References