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Short communication

Electrochemical detection of cytosine and 5-methylcytosine on Au(111) surfaces

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ABSTRACT

In this communication we report a voltammetric study of the adsorption–desorption of cytosine (C) and methylcytosine (mC) on well-defined gold (Au) electrodes. The voltammetric measurements clearly indicate that these processes are extremely sensitive to the Au surface structure and in particular to the presence of (111) surface domains. Interestingly, on Au(111) surfaces, a linear correlation between the C and mC concentrations (logarithm scale) and the peak potential of the main voltammetric feature is found. In addition, in the simultaneous presence of both molecules, mC governs the electrochemical response, which has allowed its accurate quantification in C-mC mixtures. *In situ* FTIR spectroscopic measurements have been carried out to deepen on this mC electrochemical sensitivity. This research may contribute to the future development of an electrochemical sensor for the determination of the degree of methylation in DNA.

1. Introduction

DNA methylation [1] is an epigenetic process that affects the regulation of gene expression thus playing an important role in serious human diseases such as tumours [2–4] and sterility [5,6], among others. Consequently, the development of analytical procedures for its detection and quantification is a matter of outstanding significance. In this sense, and despite some methods are already available [7–11], most of them are time and DNA material consuming. Therefore, novel, fast, sensitive, simple, and economical methods for DNA methylation assays are still sought. Due to its high sensitivity, rapid response and ease of implementation, voltammetric techniques are very promising for DNA studies [12–15]. Thus, some approaches using glassy carbon [12,16], boron doped diamond [14,17], nanocarbon film [15,18], screen printed graphite [13,19] and graphene oxide [20,21] based electrodes have been already reported for the electrochemical detection of free DNA bases, nucleosides, nucleotides and oligonucleotides under different experimental conditions.

On the other hand, it is also known that DNA bases (nucleobases) can be adsorbed on some metal solid surfaces, particularly, on Au electrodes [22–30]. This adsorption–desorption process has been also shown to be sensitive to the surface structure of the Au electrode, that is, to its specific surface atomic arrangement. Taking into account all these aspects, this contribution aims to evaluate the adsorption–desorption properties of cytosine (C) and methylcytosine (mC) on well-defined Au surfaces by cyclic voltammetry and infrared spectroscopy.

2. Experimental

Gold single crystal electrodes were prepared from small (2–3 mm diameter) crystal beads as reported previously [31]. Larger electrodes (4–5 mm in diameter) were used for the spectroelectrochemical experiments. Prior to each electrochemical experiment, the single crystal electrodes were flame-annealed and quenched with ultrapure water (Milli-Q 18.2 MΩ cm). A gold bead fully immersed in the electrochemical cell solution was also used as a polyoriented gold electrode. The quality of the gold electrodes was verified by cyclic voltammetry in 0.1 M phosphate buffer solution (pH = 7) [32]. The phosphate buffer solution was prepared using a certain ratio of NaH_2PO_4 and Na_2HPO_4 (Panreac 99% purity). The pH of the solution was checked with a Crison 507 pH-meter. Cytosine (C) and 5-methylcytosine (mC) were obtained at the highest analytical grade available (Sigma Aldrich) and were used as received. C and mC solutions were prepared in 0.1 M phosphate buffer pH 7 solution.

Voltammetric experiments were carried out in a standard three-electrode electrochemical cell. The electrode potential was controlled by a PGSTAT302N (Metrohm Autolab) system. A gold wire was used as counter electrode. The potentials were measured against a reversible hydrogen electrode (RHE) connected to the cell through a Luggin capillary. Solutions were deaerated with Ar (99.999%, AlphaGaz). All experiments were performed at room temperature (22 ± 2 °C). As usual, Au

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single crystal electrodes contacted the solution through the hanging meniscus configuration.

In situ FTIR spectra were acquired with a Nicolet Magna 850 spectrometer equipped with a liquid nitrogen cooled MCT detector. The spectroelectrochemical cell was provided with a prismatic CaF_2 window bevelled at 60°. The optical path through the solution was minimised by pressing the electrode against the window as described previously [33]. Spectra shown are composed of 100 interferograms collected with a resolution of 8 cm^{-1} and p polarized light. Spectra are presented as absorbance, according to $A = -\log(R/R_0)$ where R and R_0 are the reflectance corresponding to the single-beam spectra obtained at the sample and reference potentials, respectively. All the spectroelectrochemical experiments were performed in 1 mM cytosine or 1 mM methylcytosine in phosphate buffer (pH 7) at room temperature. Potential control during the spectroelectrochemical experiments was maintained using an EG&G PARC 175 signal generator in combination with an eDAQ EA161 potentiostat, with a RHE and a gold wire as reference and counter electrodes, respectively. FTIR experiments were performed as follows: the electrode was immersed at controlled potential (1.3 V) in the working solution and the potential was kept until the electrode was pressed against the CaF_2 window. Then, at that potential, a spectrum was recorded and subsequently the potential was moved to the next one where a new spectrum was recorded. In this way, different spectra were recorded. The spectrum collected at 0.1 V was taken as the reference one.

3. Results and discussion

Fig. 1 shows the voltammetric profiles obtained with a Au bead (polyoriented surface) and with the three basal plane electrodes (Au(100), Au(110) and Au(111)) in 0.1 M phosphate buffer solution (pH = 7) in presence of C (Fig. 1A) and mC (Fig. 1B) both at 100 μM. Results obtained clearly indicate how sensitive is the adsorption desorption of both molecules to the surface structure of the Au electrodes. Thus, distinct and characteristic voltammetric signals are recorded for each surface orientation. These features are obviously different to those obtained in absence of C or mC which are known to be related to the phosphate anion adsorption–desorption on Au surfaces (Inset Fig. 1B) [32]. Noticeably, the voltammetric profiles of the polyoriented surface show the presence of multiple and well-defined contributions which can be easily assigned with those coming from the Au single crystals.

Among the different voltammetric features, the sharp peak at about 0.75 V and 0.7 V for C and mC, respectively, obtained with the Au(111) electrode in the negative-sweep is particularly relevant. This contribution (also observed with the polyoriented surface) is clearly more intense for mC than for C for equivalent concentrations. Interestingly, when C or mC concentrations are systematically varied, a clear dependence between the potential of this peak and the concentration is observed. This dependence is depicted in Fig. 2A for mC where, for sake of comparison, the voltammetric response of a Au(111) in absence of mC is also included (dotted line). Such results exhibit that the position of the peak varies linearly with the concentration of mC (*logarithm* scale) shifting to more negative potentials and becoming more intense for increasing mC concentrations. A similar tendency is also observed for C (results not shown). However, it is worth noting that not only the peak intensity is different between mC and C but also the potential range where the peaks appear and the distinct slopes of the plot Ep vs concentration, being higher, in absolute value, for mC (0.063 V dec^{-1}) than for C (0.0388 V dec^{-1}).

Subsequently, additional experiments were performed in which the concentration of mC was systematically varied in presence of a constant

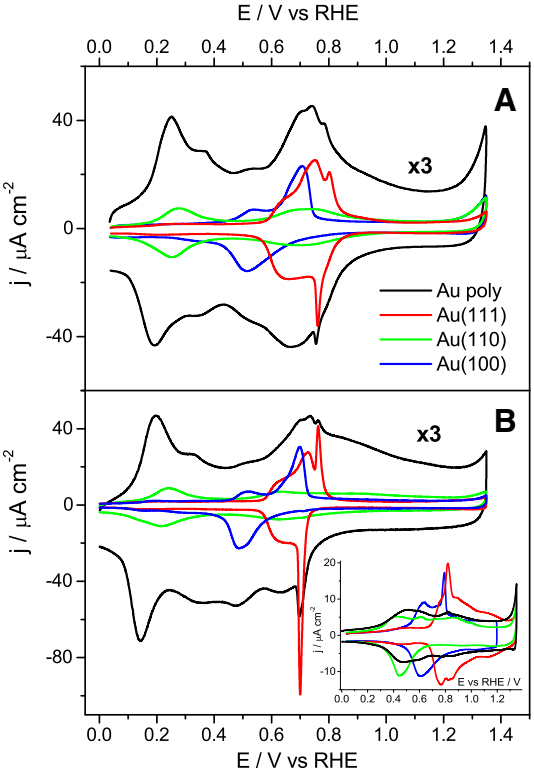

Fig. 1. Voltammetric response for cytosine (A) and 5-methylcytosine (B) adsorption–desorption ([C] = [mC] = 100 μM) on polyoriented gold, Au(111), Au(110) and Au(100) single crystals in 0.1 M phosphate buffer pH = 7 solution. Inset displays the blank voltammetric profiles. Scan rate: 50 mV s^{-1}. For sake of comparison, the response of the polyoriented Au surface is multiplied by a factor of 3 (except in the inset).

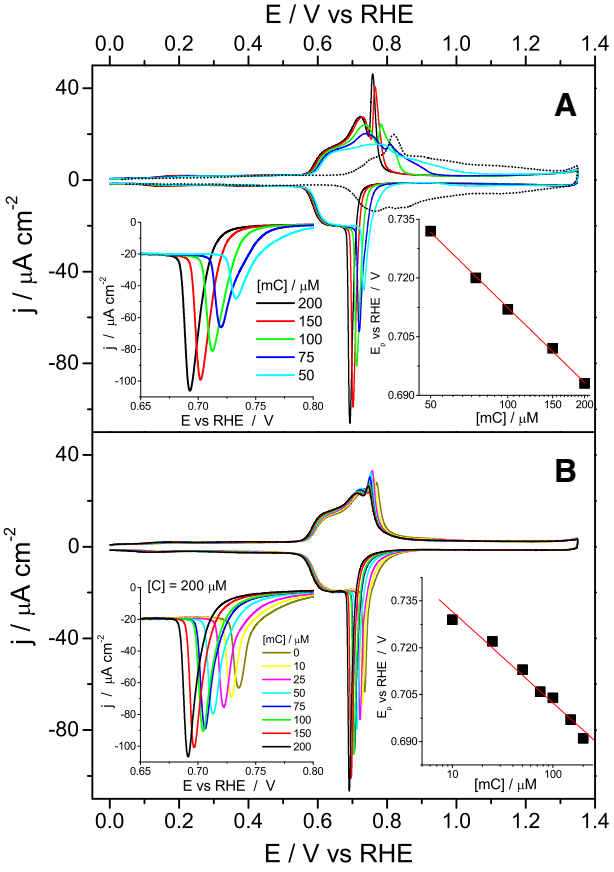

Fig. 2. Voltammetric response of a Au(111) in a 0.1 M phosphate buffer pH = 7 solution with increasing amounts of mC in absence (A) and presence of 200 μM of C (B). Black dotted line in (A) corresponds to the blank voltammogram. In both cases, a zoomed view of the spike and a peak potential vs [mC] (*log* scale) plot are shown. Scan rate: 50 mV s^{-1}.