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Scanning probe microscopic imaging of guanine on a highly oriented pyrolytic graphite electrode

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Abstract

Guanine adsorbed onto a highly oriented pyrolytic graphite electrode was studied by MAC-Mode Atomic Force Microscopy (AFM), and the electrochemical behaviour of the guanine layer was investigated with Electrochemical AFM. Guanine adsorbs spontaneously, without forming a well-packed structure, into nucleation spots, which are stable with time and cover the surface uniformly and almost completely. The process of guanine adsorption and nucleation can be controlled and the effect of altering the exposure time and varying the potential was investigated.

Keywords: Guanine; Adsorbtion; Pyrolytic graphite; Atomic force microscopy

1. Introduction

The imaging of guanine adsorbates onto solid surfaces at the molecular level has been studied using AFM and STM, but problems were encountered due to guanine's weak interaction with the substrate [1,2]. However, MAC Mode AFM permits the visualisation of molecules that are weakly attached to the surface. Electrochemical studies with guanosine, the guanine nucleotide, at concentrations near saturation led to the detection of dimers and trimers within the oxidation products [3]. The oxidation pathways of guanine and guanosine are very similar. Both oxidise at the C_8–H position by a two-step mechanism involving the loss of $4H^+$ and $4e^-$ leading to, respectively, 8-oxyguanine and 8-oxyguanosine, which are also electroactive [3,4]. The formation of oligomeric products in the electrooxidation of guanine was questioned due to its low solubility (20–25 times less than guanosine), and in the electrolysed guanine solutions, it has not been possible until now to isolate and identify oligomers as products of guanine oxidation.

This paper presents the results from in situ MAC Mode AFM imaging in electrochemically controlled conditions of adsorbed guanine at highly oriented pyrolytic graphite electrode.

2. Experimental

Guanine was from Sigma, and saturated solutions, $\sim 10^{-3}$ M, in pH 4.5 0.2 M acetate buffer, were prepared in high-purity water from a Millipore Milli-Q system (resistivity, 18 MΩ cm).

Atomic Force Microscopy (AFM) was performed with a Pico SPM controlled by a MAC Mode module and interfaced with a PicoScan controller, all from Molecular Imaging. Electrochemical control was done with a potentiostat/galvanostat PicoStat™. Silicon type II MAClevers of 225-μm length, 2.8 N m^{-1} spring constant, and 27–30 kHz resonant frequencies in liquid (Molecular Imaging) were used. Highly oriented pyrolytic graphite (HOPG), grade ZYH, from Advanced Ceramics, as working electrode, a Pt wire counter electrode, and a silver wire reference electrode were used. Freshly cleaved HOPG substrate was examined by cyclic voltammetry in acetate buffer and imaged by AFM before experiments. All images were taken at room temperature, at a scan rate of 1.95 lines s^{-1}.

3. Results and discussions

Free adsorption of guanine molecules with the electrochemical cell at open circuit was investigated. The adsorption occurred over a period of 5 min. In situ MAC Mode AFM images were obtained, Fig. 1A, and confirmed the

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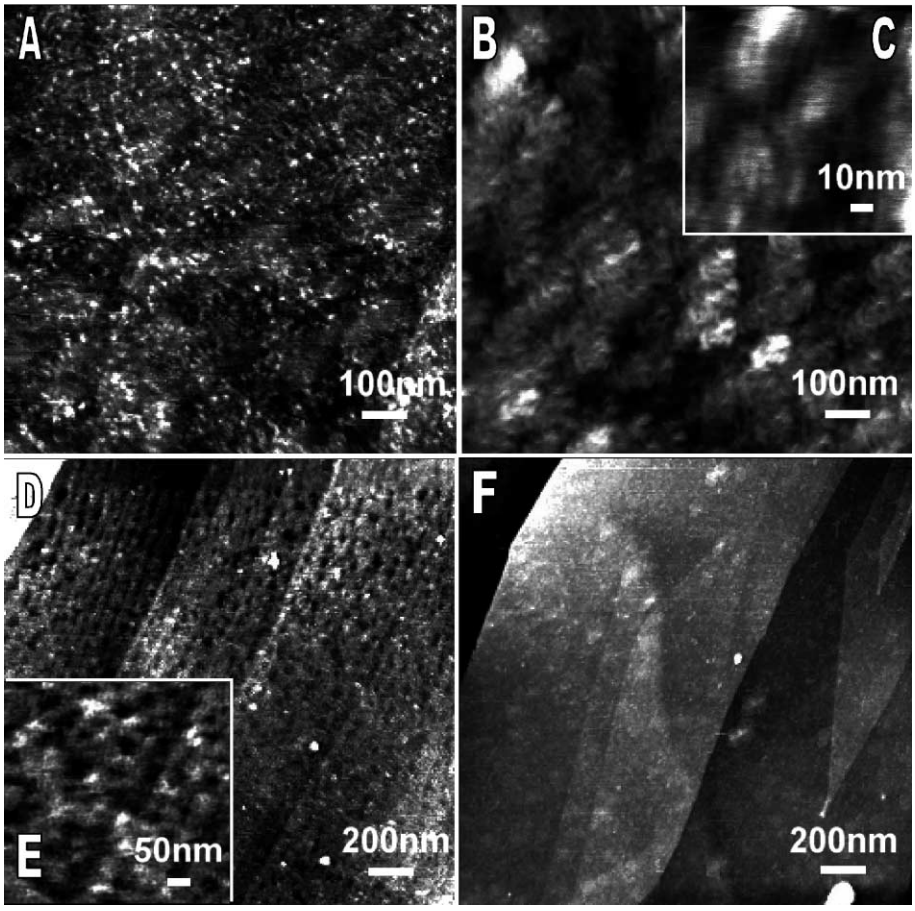

Fig. 1. In situ MAC Mode AFM images in pH 4.50 0.2 M acetate buffer of guanine adsorbed onto HOPG from a saturated guanine solution: (A) after 5 min free adsorption; (B) after 1 h free adsorption; (C) inside image (B). (D) Beginning of dissolution of the guanine layer after one cyclic voltammogram, from 0 to +1.3 V vs. Ag wire, scan rate 0.05 V s^{-1}; (E) inside image (D); (F) complete dissolution of guanine film after performing cyclic voltammetry for 5 min.

capacity of guanine molecules to spontaneously adsorb at the HOPG surface. The molecules condensed in small nuclei of 20–40-nm diameter and 2–3-nm height, corresponding to several hundred guanine molecules lying flat on the surface, covering uniformly the HOPG, without forming a well-packed structure. The effect of altering the time of exposure was explored using periods from 5 min to 1 h and the guanine layer appeared to reorganise over time. After an exposure of 1 h, Fig. 1B, the surface was better covered with guanine. The nuclei grew in islands of ~100-nm diameter and 3–6-nm height with a non-compact structure—inside the islands small nuclei of 20–40 nm were observed, Fig. 1C. Performing one cyclic voltammetric scan, the guanine layer started to dissolve and small pits of ~30-nm diameter and 2–3-nm depth appeared, Fig. 1D, and the guanine layer completely dissolved after 5 min, Fig. 1F.

The influence of the HOPG potential in the process of guanine adsorption was investigated. First, five successive cyclic voltammograms, from 0 to +1.3 V (vs. Ag wire) scan rate 0.1 V s^{-1}, were recorded in a guanine solution. After the HOPG was held for 5 min at +0.75 V, the oxidation potential of guanine, condensation of guanine into larger nuclei of 90–150-nm diameter and 10–30-nm height occurred. The nuclei grouped in intercalated polymer-like chains of different lengths, some longer than 1 μm, uniformly distributed on the HOPG surface, Fig. 2A–C.

The AFM images of adsorbed guanine molecules from a saturated solution during controlled oxidation support the conclusion that the guanine oxidation can induce the formation of oligomers at the HOPG electrode.

The oligomers are stacked at the surface together with the guanine molecules and other oxidation products forming the polymer chain. All the components must interact between themselves and with the HOPG surface by hydrogen bonding, London dispersion forces, and hydrophobic interactions. Due to these weak forces, when a cyclic voltammogram was run in the guanine solution, it was observed that all polymer-like chains dissolved at ~0.9 V, due to desorption of the guanine oxidation products, Fig. 2D and E.

4. Conclusion

The process of guanine adsorption and nucleation at the HOPG electrode is dependent on the potential of the electrode. In situ images of guanine adsorbates demonstrate

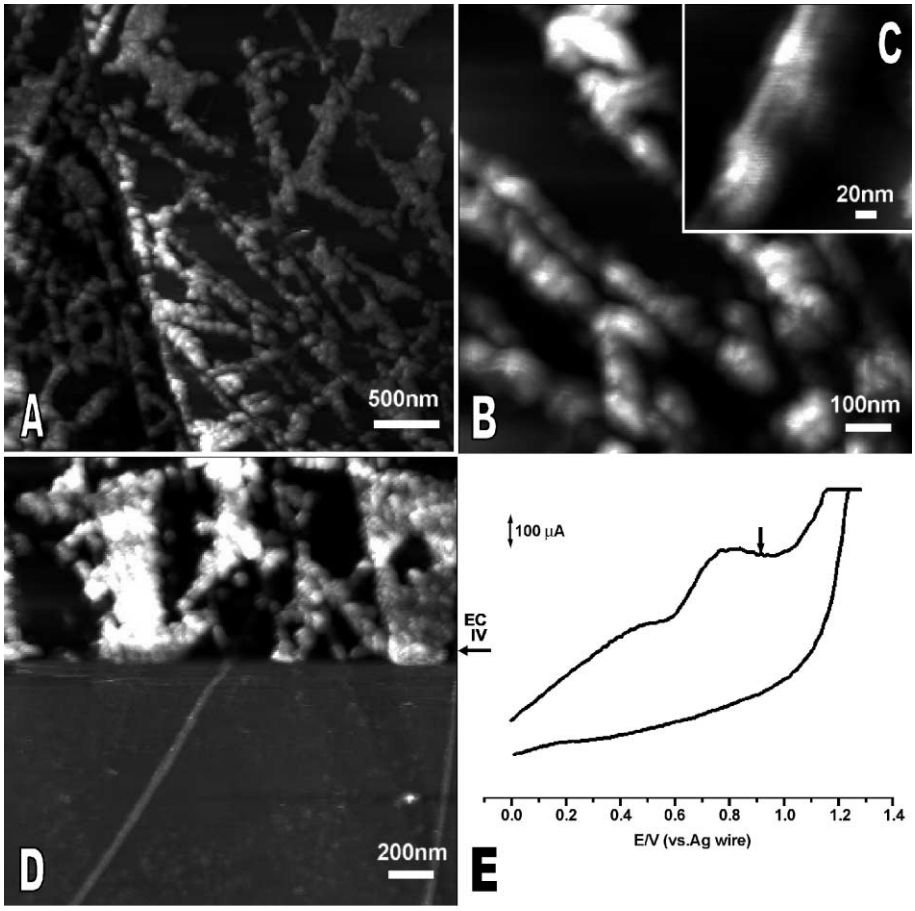

Fig. 2. In situ MAC Mode AFM images in pH 4.50 0.2 M acetate buffer of guanine adsorbed onto HOPG from a saturated guanine solution: (A) after five cyclic voltammograms (CV), from 0 to +1.3 V, scan rate 100 mV s^{-1}; (B) after 5 min at +0.75 V; (C) inside image (B). (D) The applied potential +0.75 V was changed during imaging while running a CV (E) and at ~0.9 V, all polymer-like chains were dissolved due to desorption of guanine oxidation products.

that MAC Mode AFM is a powerful tool to investigate the molecules attached to the surface by soft forces such as London dispersion forces and hydrophobic interactions.

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