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Nucleosides and ODN electrochemical detection onto boron doped diamond electrodes

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Abstract

Boron doped diamond (BDD) is a promising material for electroanalytical chemistry due mainly to its chemical stability, its high electrical conductivity and to the large amplitude of its electroactive window in aqueous media. The latter feature allowed us to study the direct oxidation of the two electroactive nucleosides, guanosine and adenosine. The BDD electrode was first activated by applying high oxidizing potentials, allowing to increase anodically its working potential window through the oxidation of CH surface groups into hydroxyl and carbonyl terminations. Guanosine (1.2 V vs. Ag/AgCl) and adenosine (1.5 V vs. Ag/AgCl) could then be detected electrochemically with an acceptable signal to noise ratio. The electrochemical signature of each oxidizable base was assessed using differential pulse voltammetry (DPV), in solutions containing one or both nucleosides. These experiments pointed out the existence of adsorption phenomena of the oxidized products onto the diamond surface. Scanning electrochemical microscopy (SECM) was used to investigate these adsorption effects at the microscopic scale. The usefulness of BDD electrodes for the direct electrochemical detection of synthetic oligonucleotides is also evidenced.
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1. Introduction

The last decades have seen the emergence of a tremendous interest for DNA detection for medical, pharmaceutical or environmental applications. Several techniques have been proposed, such as fluorescence microscopy or direct electrochemical oxidation of electroactive nucleic bases [1]. Indeed, guanine and adenine can be oxidized at carbon electrodes, but at rather high anodic potentials ($E_{guan} = 0.81$ and $E_{ad} = 1.4$ V/SCE) [2]. Consequently, DNA detection has been performed mostly on the basis of guanosine oxidation, leading to strongly sequence-sensitive results and to a lack of quantitative information.

The simultaneous direct oxidation of both guanosine and adenosine may overcome these difficulties. However, this requires the judicious choice of the recording method [3] or/and of the electrochemical interface under investigation. Boron doped diamond (BDD) appears as an interesting material owing to its wide potential window with low background current in aqueous media, and we want to show here the usefulness of BDD electrodes for direct oxidation of oligonucleotides (ODN). For this purpose, we have investigated the electrochemical behavior of guanosine and adenosine on such electrodes using differential pulse voltammetry (DPV). These nucleosides were utilised here as models of DNA electroactive sites. Our results show strong adsorption behaviors of the oxidation products of both nucleosides on the overall interface. Scanning electrochemical microscopy (SECM) provided insights in the kinetics of the aforementioned phenomena. Finally, preliminary results dealing with the direct detection of synthetic oligonucleotides on BDD are presented.

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2. Experimental

2.1. Reagents

Nucleosides were obtained from Aldrich and used as-received without purification. Stock solutions (10 mM) of the bases were prepared in 0.1 M phosphate buffer pH 7. Synthetic ODN (sequence TGC ATA CG), purchased from Apibio, were purified by HPLC and obtained in the lyophilised form. Prior to any experiment, ODN were dissolved in 0.1 M phosphate buffer pH 7 to obtain 1.5 mM stock solutions.

2.2. Electrochemical procedures

BDD films on p-type silicon wafers (10 mm in diameter) were prepared by means of microwave plasma-assisted CVD, as described elsewhere [4]. The obtained boron level within the diamond films is estimated to be approximately 10^{20} atoms cm^{-3}. The electrochemical measurements were performed in a three-electrode one-compartment Teflon cell (600 μl), the BDD film constituting the working electrode. The counter electrode was a Pt wire, and a KCl saturated Ag/AgCl electrode was used as potential reference. For these experiments, a computer-driven PAR 283 potentiostat (EGG/PAR) was used.

Cyclic voltammetries (CV) were performed in 0.1 M phosphate buffer pH 7 by scanning the potential between -1.7 and $+2$ V vs. Ag/AgCl at a scan rate of 50 mV s^{-1}. Differential pulse voltammetries (DPV) were recorded in the potential interval 0 to $+2.1$ V vs. Ag/AgCl; the DPV parameters were as follows: pulse amplitude, 25 mV; pulse width, 60 ms; scan rate, 22.2 mV s^{-1}.

2.3. SECM procedure

The SECM apparatus was built at LEPMI in collaboration with J. Heinze, and is nearly identical to the one described in Ref. [5]. The SECM tips were made of a Pt wire (Goodfellow, 10 μm in diameter), sealed in a glass tube (Brand). The ratio (Rg) of the glass ring radius to the Pt disk radius was 25. In this case, the electrolyte was a solution of 0.5 M Na_2SO_4 and 10 mM $K_3Fe(CN)_6$ in water. Positive feedback mode was used to assess the adsorption phenomena: the BDD substrate was anodically polarized, the probing tip was cathodically polarized, in order to reduce hexacyanoferrate (III) at the tip and regenerate it at the BDD electrode.

3. Results and discussion

BDD films as-obtained after plasma deposition exhibit a CH-terminated surface. To obtain an anodically extended stable electroactive window, the BDD electrode needs to undergo a pre-oxidation treatment in aqueous electrolyte,

which derivatises the CH terminations into oxygen-terminated groups [6] (e.g., hydroxyls and/or carbonyls). The electrochemical oxidation treatment consisted here of polarizing the electrode at a potential of $+2.4$ V vs. Ag/AgCl in 0.1 M KH_2PO_4 for 1 h [6]. The electrode surface turns on the one hand from hydrophobic to hydrophilic, and on the other hand becomes more suitable for redox systems with high oxidation potentials, compared to the as-deposited diamond electrode. However, it should be noted that this strong oxidation increases the electron transfer resistance for various redox species such as hexacyanoferrate by increasing the potential barrier for electron transfer [7].

The voltammograms of the as-deposited and electrochemically oxidized diamond electrodes are shown in Fig. 1.

They correspond perfectly to the state-of-the-art known voltammograms for such electrodes, including the two irreversible oxidation waves observed at 1.55 and 1.9 V vs. Ag/AgCl which are linked to the oxidation of the diamond surface [8] but are still not clearly explained. After oxidation, the BDD potential window is extended in the anodic domain from 1.4 to 1.9 V vs. Ag/AgCl, which permits electrochemical detection of guanosine and adenosine. Moreover, the recorded background current for oxidized BDD is low compared to the one obtained with other classical carbon substrates. This allowed to assess the electroanalytical properties of oxidized BDD with regards to guanosine and adenosine oxidation. Fig. 2 shows the DPV signals obtained for pure solutions of guanosine (curve a) and adenosine (curve b) and for a mixture of the two species in equimolar proportions (curve c).

Each nucleoside shows a well-defined peak, at potentials around 1.17 and 1.5 V vs. Ag/AgCl, respectively, for guanosine and adenosine. Surprisingly, we observed an important decrease of the adenosine peak amplitude in the mixture compared to the one in a pure solution of the nucleoside: the oxidation current of adenosine was roughly divided by a factor of 8 while the current recorded for guanosine oxidation remained stable. This may indicate a strong adsorption either of guanosine, which acts as limiting step in the reaction process, or of the 8-oxo-guanosine, the
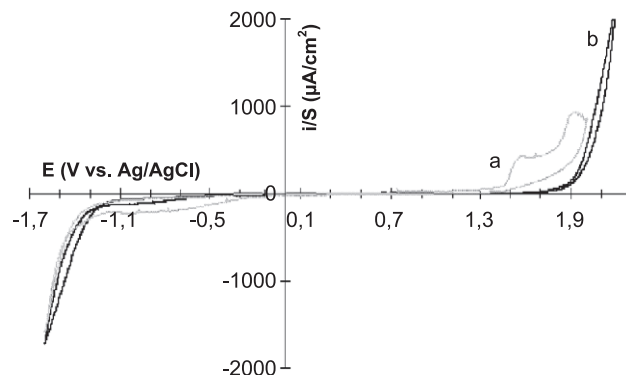

Fig. 1. Cyclic voltammetric curves for the (a) as-deposited and (b) electrochemically oxidized diamond surfaces in 0.1 M phosphate buffer pH 7 at a sweep rate of 50 mV s^{-1}.
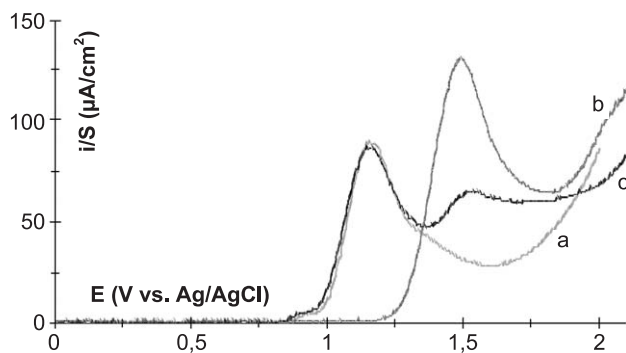
Fig. 2. Differential pulse voltammetric curves in a solution of 2.5 mM (a) guanosine, (b) adenosine, (c) both guanosine and adenosine in 0.1 M phosphate buffer pH 7 on an oxidized BDD electrode.

expected product of the guanosine oxidation, which hinders either the charge transfer to the adenosine or the matter transfer of the latter to the electrode surface.

To assess the performances of our system for nucleosides sensing, we established calibration curves for both species in pure solutions (Fig. 3). The detection limits for guanosine and adenosine were obtained for concentrations of 10 and 50 µM. It is important to note that, if the obtained detection limits on glassy carbon electrodes are similar, the ratio between peak current and background current is more important for BDD electrodes. For example, this ratio is around 3.2 for diamond and 1.3 for glassy carbon in a solution of 50 µM adenosine. This underlines the reliability of BDD electrodes for nucleosides detection owing to its enlarged potential window and its low and stable background current.

However, the signal amplitude does not vary linearly with the nucleosides concentration as displayed in Fig. 3. These results suggest the existence of nucleosides or oxidation products adsorption phenomena.

In order to discriminate between nucleoside and oxidation product adsorption, we pre-polarized the BDD electrode (at 1.2 and 1.55 V vs. Ag/AgCl in guanosine and adenosine solutions, respectively), for durations increasing from 0 to 900 s, before performing the DPV experiments. These potentials were chosen to promote nucleosides oxi-
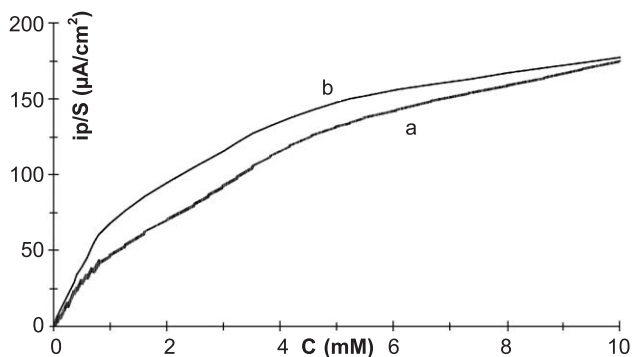

Fig. 3. Evolution of i_{peak} with (a) guanosine and (b) adenosine concentration in 0.1 M phosphate buffer pH 7.
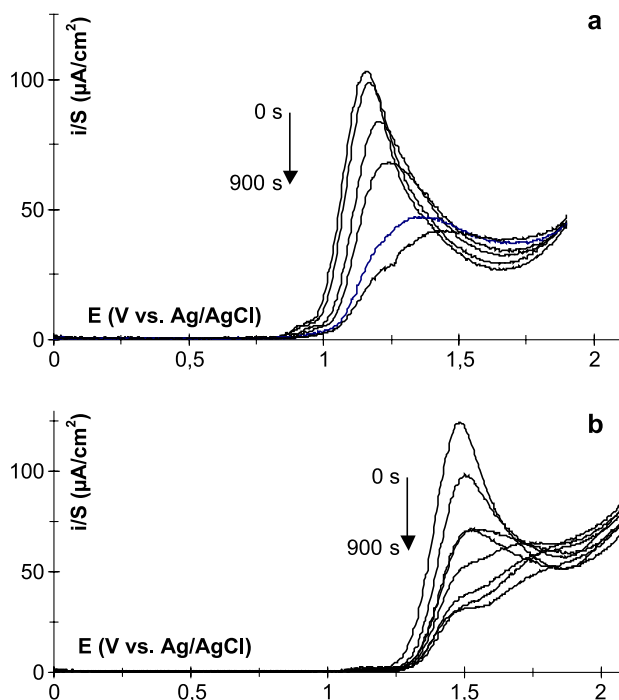

Fig. 4. Evolution of the differential pulse voltammograms of 2.5 mM (a) guanosine and (b) adenosine in 0.1 M phosphate buffer pH 7 on oxidized BDD electrode with increasing polarization durations (from 0 to 900 s) at potentials of (a) 1.2 and (b) 1.55 V vs. Ag/AgCl.

dation. Thereby, in the case of the adsorption of the reaction products, these pre-polarizations should influence the DPV responses of the nucleoside in solution. Fig. 4 shows the impact of increasing polarization durations onto the DPV responses of guanosine (curve a) and adenosine (curve b).

For each nucleoside, the pre-polarization affects the DPV peak amplitude, which decreases dramatically with increasing polarization duration. For example, in the case of adenosine, the DPV signal is lowered by 21% after 10 s polarization and 43% after 60 s. This indicates clearly that
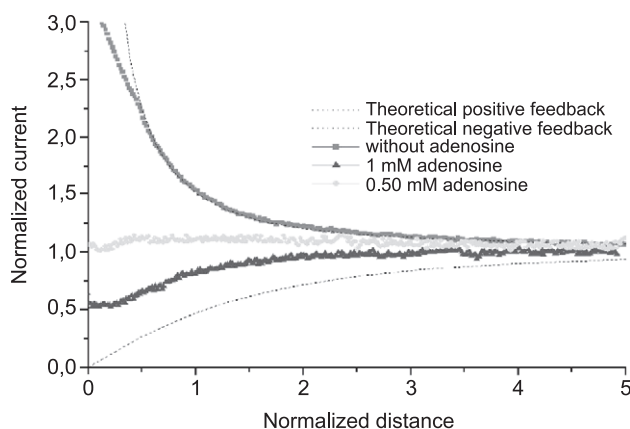

Fig. 5. Approach curves of BDD surface prior and after polarization in adenosine solutions containing 1 and 0.5 mM of nucleoside. Polarization conditions as in Fig. 4. Scan rate: − 1 µm s⁻¹. E_{tip} = − 0.4 V vs. Ag/AgCl and E_{BDD} = 1.05 V vs. Ag/AgCl.

the major adsorption phenomenon is due to the nucleosides' oxidation products.

Otherwise, for guanosine, the oxidation peak is enlarged and displaced toward more anodic potentials with increasing polarization durations. It also seems that for long pre-polarization times, the guanosine oxidation peak separates into two different peaks. The latter trend is more visible in the case of adenosine: after a polarization of 120 s, the oxidation peak splits into two peaks, one at the adenosine oxidation potential mentioned before, and the second one which shifts to more anodic potentials with increasing polarization time. Indeed, nucleosides oxidation is a rather complex phenomenon which implies multi-step reactions involving electron and proton transfers [9]. One of these steps may be hindered by the electrode fouling consecutive to the adsorption of the nucleosides oxidation products, leading to peak splitting. Nevertheless, such phenomena remains unclear and complementary experiments are currently on the way.

The latter DPV experiments reveal adsorption phenomena through macroscopic effects. We tried to obtain more localized information by characterizing the BDD/electrolyte interface by SECM. First, 2-D scans (not shown here) of the BDD electrode at constant height were performed in the positive feedback mode using hexacyanoferrate (III) as mediator to obtain a microscopic visualization of the adsorption phenomena. Briefly, prior to the oxidation of the nucleosides, the surface scans showed an inhomogeneous interface reactivity, which is likely to be related to heterogeneities in the BDD morphology. After pre-polarization, 2-D scans exhibit a more homogeneous electrode reactivity, which however, is lowered by an average factor of 15%. Moreover, this loss of reactivity is influenced by the nucleoside concentration as well as by the polarization time, and particularly, is negligible when no pre-polarization is applied.

Second, approach curves on the BDD surface were recorded prior to and after pre-polarization of the electrode in guanosine or adenosine solutions for different concentrations of the both species. Fig. 5 displays the results obtained prior to and after polarization at 1.55 V vs. Ag/AgCl during 900 s in adenosine solutions of 1 and 0.5 mM. Similar results were obtained for guanosine solutions. Prior to the oxidation of nucleosides, the approach curve follows a classical positive feedback shape that fits the model curve perfectly corresponding to fast mediator regeneration. After polarizing the interface in the presence of adenosine, the approach curves do change from pure positive feedback to more or less pronounced negative feedback. Also, the shift to negative feedback is more pronounced at higher adenosine concentrations. Such a behavior is likely to be linked to a loss of surface reactivity due to the adsorption of oxidation products. We are currently trying to model these phenomena on the basis of two different hypotheses: (i) nucleoside oxidation generates a film at the BDD surface and the mediator (or the non-oxidized nucleoside) diffuses through the film thickness from the solution to the electrode (this
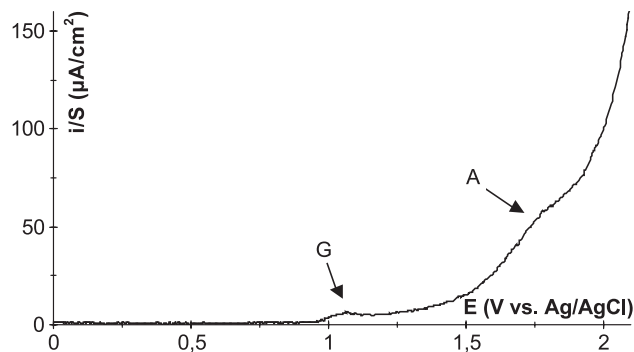

Fig. 6. DPV in a solution of 500 μM ODN (sequence TGC ATA CG) in 0.1 M phosphate buffer pH 7 on oxidized BDD electrode.

film then acts as a porous membrane); (ii) oxidation products are adsorbed onto specific sites and the BDD surface acts as a blocking electrode.

4. Conclusion

BDD electrodes have been used for the electrochemical detection of nucleosides. The original properties of the BDD (low background current, wide potential window in aqueous media) allowed to electrochemically oxidize guanosine and adenosine. By means of SECM and DPV experiments, we have highlighted adsorption phenomena of the nucleosides oxidation products. Future work will deal with ODN detection on BDD electrode (preliminary results are shown in Fig. 6).

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