**Lipoic acid–palladium complex interaction with DNA, voltammetric and AFM characterization**

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**A B S T R A C T**

The mechanism of interaction of lipoic acid–palladium complex (LAPd) with double-stranded DNA (dsDNA), as well as the adsorption process and the redox behaviour of LAPd, of its ligand lipoic acid (LA), and of the LAPd-containing dietary supplement, Poly-MVATM, were studied using atomic force microscopy (AFM) and voltammetry at highly oriented pyrolytic graphite (HOPG) and glassy carbon electrodes. In the presence of small concentrations of LAPd molecules, the dsDNA molecules appeared less knotted and bended, and more extended on the HOPG surface, when compared with the dsDNA molecules adsorbed from the same dsDNA solution concentration. The voltammetric results demonstrated the interaction of both LAPd and Poly-MVATM with dsDNA, but no oxidative damage caused to dsDNA was detected. AFM images revealed different adsorption patterns and degree of surface coverage and correlation with the structure, the concentration of the solution, the applied potential, and the voltammetric behaviour of the LA, LAPd and Poly–MVA TM was observed. The application of a negative potential caused the dissociation of the LAPd complex and Pd(0) nanoparticle deposition, whereas the application of a positive potential induced the oxidation of the LAPd complex and the formation of a mixed layer of LA and palladium oxides.

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1. Introduction

The lipoic acid–palladium complex (LAPd) was originally designed as a non-toxic chemotherapeutic agent, in a prescription version called DNA Reductase, and consists of a palladium bonded to both end-groups of a lipoic acid (LA), the two sulfurs of the thiolane ring and the carboxyl of the pentanoic chain in a 1:1 ratio, Scheme 1 [1,2]. This arrangement is unique in that it allows the molecule to be both water and lipid soluble. The presence of the free radical scavenger, LA [3,4], and the addition of an alternative energy source, palladium [2,5,6], led to consider that LAPd can be used in the treatment of various cancers [2,7,8].

The LA, ligand in the LAPd complex, is a well established antioxidant that may act as a buffer in cancer therapy [9] where the drugs used are known promoters of oxidative stress, or in therapy of diseases associated with oxidative stress [10–13], either directly as a free radical scavenger [14–16] or indirectly due to its synergistic action with other antioxidants [17].

The LAPd-containing liquid dietary supplement, Poly-MVA TM, is based on a LAPd polymer that exists as a trimer of lipoic acid–palladium complex joined to thiamine [1]. Besides the LAPd core unit, Poly-MVA TM also contains free LA [18], minerals (molybdenum, rhodium, ruthenium), vitamins (B1, B2, B12) and amino acids (N-acetyl cysteine and formyl methionine). LAPd in Poly-MVA TM was shown to regulate ischemic cell death and may be a potent neuroprotective agent for victims of transient ischemic attack, cardiac arrest, anesthetic accidents, or drowning [19]. Toxicological studies showed no accumulation in or damage to any tissues, and the median lethal dose, LD50, in mice is greater than 5000 mg kg−1 (the highest dosage tested) [19–21]. The Ames test confirmed that the complex is free of mutagenicity [22].

Voltammetric methods have been used for the characterization of LA and palladium(II) containing solutions, at a glassy carbon electrode (GCE) [18,23]. The same methods were employed for the determination LA in two dietary supplements, Poly-MVA TM and Solgar Alpha-Lipoic Acid [18]. Atomic force microscopy (AFM) was used to investigate the surface topography of highly oriented pyrolytic graphite (HOPG) electrodes modified with electrodeposited palladium nanoparticles and nanowires [23].

In the present paper, the mechanism of interaction of LAPd with double-stranded DNA (dsDNA) was studied voltammetrically and morphologically evaluated by magnetic AC mode (MAC Mode) AFM. For this purpose, a systematic study of the adsorption process and the redox behaviour of LAPd, of the ligand LA and of the LAPd-containing Poly-MVA TM was carried out at two carbon electrodes, HOPG and GCE. The results revealed important correlations

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between the different adsorption patterns, conformational changes and the voltammetric behaviour of LAPd and dsDNA upon interaction.

2. Experimental

2.1. Materials and reagents

Solutions of 10 mM LAPd and 13 mg mL\(^{-1}\) Poly-MVATM (of proprietary blend as marketed, containing 40 mM LAPd) were from Garnett McKeen Laboratory, Inc. and were used without further purification. Solutions of different concentrations of either LAPd or Poly-MVATM were freshly prepared before each experiment by dilution of the appropriate quantity in supporting electrolyte. DL-\(\alpha\)-lipoic acid (LA) was purchased from Sigma-Aldrich. A stock solution of 100 mM LA was prepared in ethanol/water 1:1 and kept at 4°C. Calf thymus dsDNA and all the other reagents were Merck analytical grade. A stock solution of 300 \(\mu\)g mL\(^{-1}\) dsDNA was prepared in deionized water and kept at 4°C.

The solutions were then diluted to the desired concentration by mixing buffer supporting electrolyte. All solutions were prepared using analytical grade reagents and purified water from a Millipore Milli-Q system (conductivity \(\leq 0.1\ \mu\)S cm\(^{-1}\)). The supporting electrolyte solutions were 0.1 M phosphate buffer pH 7.0. Nitrogen saturated solutions were obtained by bubbling high purity N\(_2\) for a minimum of 10 min and the voltammetric behaviour of LAPd and dsDNA upon interaction.

2.2. Atomic force microscopy

HOPG grade ZYB of 15 mm \(\times\) 15 mm \(\times\) 2 mm dimensions, from Advanced Ceramics Co., was used as a substrate in the AFM study. The HOPG was freshly cleaved with adhesive tape prior to each experiment and imaged by MAC mode AFM in order to establish its cleanliness.

AFM was performed with a PicoSPM controlled by a MAC Mode module and interfaced with a PicoScan controller from Agilent Technologies, Tempe, AZ (formerly Molecular Imaging). A CS AFM S scanner, with a scan range 6 \(\mu\)m in \(x\)–\(y\) and 2 \(\mu\)m in \(z\), and silicon type II MAClevers of 225 \(\mu\)m length, 2.8 N m\(^{-1}\) spring constants, 60–90 kHz resonant frequencies in air, from Agilent Technologies, were used. All images (256 samples line\(^{-1}\) \(\times\) 256 lines) were topographical and were taken in air at room temperature, by MAC Mode, with scan rates of 0.8–2.0 lines s\(^{-1}\). When necessary, the AFM images were processed by flattening in order to remove the background slope and the contrast and brightness were adjusted.

Section analyses were performed with PicoScan software version 5.3.1. Agilent Technologies and with Origin version 6.0 from Microlab Software, Inc., USA. The mean values of the heights and standard deviations were calculated with Origin version 6.0 from Microlab Software, Inc., USA.

2.3. Voltammetric parameters and electrochemical cells

The voltammetric experiments were performed using an Autolab running with GPES 4.9 software, Eco-Chemie, Utrecht, The Netherlands. Cyclic voltammograms (CVs) were recorded at scan rate of 50 mV s\(^{-1}\). The differential pulse voltammetry (DPV) conditions were: pulse amplitude 50 mV, pulse width 70 ms, step potential 2 mV and scan rate of 5 mV s\(^{-1}\). Measurements were carried out in a 0.5 mL one-compartment electrochemical cell using a glassy carbon electrode (GCE) \((d = 1.5\ mm)\), with a Pt wire counter electrode, and a Ag/AgCl (3 M KCl) electrode as reference.

The GCE was polished using diamond spray (particle size 1 \(\mu\)m) before every electrochemical experiment. After polishing, the electrode was rinsed thoroughly with Milli-Q water then it was sonicated for 1 min in an ultrasound bath and again rinsed with water. After this mechanical treatment, the GCE was placed in buffer electrolyte and various CVs were recorded at \(V = 100\ mV\ s\^{-1}\) until a steady state baseline voltammogram was obtained. This procedure ensured very reproducible experimental results.

Electrochemical deposition of LAPd on the HOPG electrode surface was performed in a one-compartment Teflon cell of approximately 12.5 mm internal diameter, holding the HOPG working electrode on the bottom of the cell. The Pt wire counter and the Ag/AgCl reference electrodes were placed in the cell dipping approximately 5 mm into the solution.

2.4. Sample preparation

The adsorption of LA, LAPd and Poly-MVATM onto HOPG and GCE and the dsDNA interaction with LAPd and Poly-MVATM were studied by MAC mode AFM in air and voltammetric methods, using the procedures described below. For all modified HOPG and GCEs the excess of solution was removed with Millipore Milli-Q water and the electrodes dried in a sterile atmosphere prior to AFM and voltammetric measurements.

2.4.1. Procedure 1 – LA, LAPd, Poly-MVATM and control dsDNA modified HOPG

The spontaneous adsorption of control dsDNA, from 5 \(\mu\)g mL\(^{-1}\) or 10 \(\mu\)g mL\(^{-1}\) dsDNA, and different concentrations of LA, LAPd and Poly-MVATM solutions was performed depositing 200 \(\mu\)L samples of the desired solutions onto freshly cleaved HOPG surfaces and incubating for 3 min.

2.4.2. Procedure 2 – electrodeposited LAPd modified HOPG

The electrodeposition of LAPd was performed from concentrations of 0.1 mM LAPd in 0.1 M phosphate buffer solutions pH 7.0, by applying \(-1.0\ V\ or\ +1.2\ V\ vs.\ Ag/AgCl\ (3\ M\ KCl)\), during 30 min.

2.4.3. Procedure 3 – electrodeposited LAPd and Poly-MVATM modified GCE

The electrodeposition was performed from 1 mM LAPd or 1.3 mg mL\(^{-1}\) Poly-MVATM (containing 4 mM LAPd) in 0.1 M phosphate buffer pH 7.0, by continuous cycling (10 CVs) or by applying one of the potentials: \(-1.00\ V\ or\ +1.20\ V\ vs.\ Ag/AgCl\ (3\ M\ KCl)\), during 30 min.
2.4.4. Procedure 4 – layer by layer dsDNA–LAPd modified HOPG
A volume of 200 μL from a 5 μM LAPd in 0.1 M phosphate buffer solution pH 7.0 was deposited for 3 min on a control dsDNA-modified HOPG electrode, Procedure 1 (5 μg mL⁻¹ dsDNA).

2.4.5. Procedure 5 – layer by layer LAPd–dsDNA modified HOPG
A volume of 200 μL from a 5 μg mL⁻¹ dsDNA in 0.1 M phosphate buffer solution pH 7.0 was deposited for 3 min on a LAPd-modified HOPG electrode obtained using either 1 μM or 0.1 μM LAPd solutions, Procedure 1.

2.4.6. Procedure 6 – dsDNA–LAPd and dsDNA–Poly-MVA TM modified HOPG
For the preparation of dsDNA–LAPd modified HOPG, 5 μg mL⁻¹ dsDNA were incubated with 5 μM LAPd or 0.1 μM LAPd, in 0.1 M phosphate buffer pH 7.0, at room temperature, during 24 h. For the preparation of dsDNA–Poly-MVA TM modified HOPG, 10 μg mL⁻¹ dsDNA were incubated with 3.3 μg mL⁻¹ Poly-MVA TM (containing 10 μM LAPd), in 0.1 M phosphate buffer pH 7.0, at room temperature, during 24 h. In order to prepare the dsDNA–LAPd and dsDNA–Poly-MVA TM modified HOPG, 200 μL samples of the respective incubated solution were placed onto the freshly cleaved HOPG for 3 min.

2.4.7. Procedure 7 – control dsDNA, dsDNA–LAPd and dsDNA–Poly-MVA TM modified GCE
The control dsDNA modified GCE was obtained by depositing a volume of 5 μL of 50 μg mL⁻¹ dsDNA in 0.1 M acetate buffer solution pH 4.5 onto the GCE for 10 min. The dsDNA–LAPd and dsDNA–Poly-MVA TM modified GCE were obtained by incubating the control dsDNA modified GCE for 3, 5 and 10 min with 1.0 mM LAPd or 1.3 mg mL⁻¹ Poly-MVA TM (containing 4 mM LAPd) in 0.1 M phosphate buffer pH 7.0.

2.4.8. Procedure 8 – control dsDNA and dsDNA–LAPd modified GCE
The control dsDNA modified GCE was prepared from a 30 μg mL⁻¹ dsDNA in 0.1 M acetate buffer solution pH 4.5. The dsDNA–LAPd modified GCE was prepared from an incubated solution containing 30 μg mL⁻¹ dsDNA and 1 mM LAPd in 0.1 M phosphate buffer pH 7.0 at room temperature, during 48 h. The control dsDNA modified GCE and the dsDNA–LAPd modified GCE were prepared depositing 3 drops of 5 μL each on the GCE and dried under a flow of pure N₂ gas. The modified GCE was then placed in the electrochemical cell where the voltammograms were always recorded in 0.1 M acetate buffer pH 4.5.

3. Results and discussion

3.1. Adsorption process and redox behaviour of LA, LAPd and Poly-MVA TM

3.1.1. AFM characterization of adsorbed LA, LAPd and Poly-MVA TM
The adsorption process of the LA, LAPd and Poly-MVA TM, was first investigated by AFM, using the procedures from Section 2.4. As described below, the topographical images clearly showed the capacity of LA, LAPd and Poly-MVA TM to interact and adsorb spontaneously on carbon electrode surfaces, forming different morphological films depending on solution concentration.

In all the AFM experiments the HOPG was used as working electrode, as its atomically flat terraces permit the correct evaluation of the morphological features of the adsorbed molecules and films. As a comparison, the GC surface has a root-mean-square (r.m.s.) roughness of 2.10 nm while the HOPG surface has a r.m.s. roughness of less than 0.06 nm, for a 1000 nm × 1000 nm surface area. Furthermore, the experiments using GCE and HOPG electrodes showed similar electrochemical behaviour.

3.1.1.1. Spontaneous adsorption of LA onto HOPG. The LA molecule is the ligand in the LAPd complex and has a disulfide-containing base, a short alkyl chain with four CH₂ units, and a carboxyl terminus [18]. The spontaneous adsorption of LA onto HOPG (Fig. 1A–D) was performed using the Procedure 1. AFM images in air of the LA modified HOPG, obtained from a solution of 400 μM LA, showed an approximately 0.5 nm height thin LA film (Fig. 1A) that corresponds to a monolayer formed by hydrophobic interactions between LA alkyl chains and the hydrophobic HOPG surface. Also the LA molecules have the tendency to form 0.8–5.0 nm height spherical aggregates which are uniformly distributed within the LA monolayer.

Using a higher concentration of 40 mM LA, two morphologically different LA layers were observed (Fig. 1B), with 1.5 ± 0.2 nm difference in height between the layers. The internal structure of those two LA layers was revealed in the higher magnification AFM images presented in Fig. 1C and D, which have the scan centres marked as white crosses in Fig. 1B. The first layer (Fig. 1C) was a thin multilayer of LA molecules, which covered completely the HOPG electrode, showed no pores and with large spherical aggregates of 1.0–6.0 nm height embedded into its structure. The second LA layer (Fig. 1D) was a thick and smooth multilayer, with only a few narrow indentations, of 0.8–1.8 nm depth, that were not sufficiently deep to reach the HOPG substrate underneath.

3.1.1.2. Spontaneous adsorption of LAPd onto HOPG. The LAPd modified HOPG electrode was obtained by spontaneous adsorption during 3 min, from different concentrations of LAPd, using the method described in Procedure 1. AFM images of the LAPd modified HOPG obtained from a low concentration of 0.1 μM LAPd solution showed large aggregates of 0.9 ± 0.2 nm height (Fig. 2A). LAPd presents a large three-dimensional structure, with the palladium in the centre of the complex, covalently coordinated with both oxygens of the LA carbonyl and one or more sulphur atoms (Scheme 1)[2]. The approximately 0.9 nm height of the LAPd aggregates is related with the deposition of a monolayer of molecules on the HOPG surface.

AFM images of adsorption from a 1 μM LAPd solution (Fig. 2B) showed looped filaments of 1.4 ± 0.1 nm height, as a result of the aggregation of the small LAPd molecules, and large portions of uncovered HOPG. AFM images of adsorption from a 10 μM LAPd solution (Fig. 2C) and 50 μM LAPd solutions (data not shown) showed similar results: a less compact LAPd film with larger pores of 1.6 ± 0.3 nm depth. The height of the LAPd films constantly increased with increasing the LAPd solution concentration, due to the formation of LAPd multilayers. Inside the LAPd film small aggregated molecules were observed.

AFM images of the LAPd modified HOPG obtained from a 100 μM LAPd solution (Fig. 2D) showed the formation of a thick uniform film, covering the electrode completely. Small indentations into the LAPd film were observed with the measured depth 0.7 ± 0.1 nm, not sufficiently deep to reach the HOPG. AFM images of LAPd modified HOPG surfaces obtained by spontaneous adsorption during 30 min
from the same solution concentration also led to a complete coverage of the HOPG.

3.1.1.3. Adsorption of LAPd under an applied potential. Electrodeposited LAPd modified HOPG surfaces were achieved by applying the potentials of −1.0 V or +1.2 V, during 30 min, in 0.1 mM LAPd, as described in Procedure 2. Negative and positive applied potentials have different effect on the adsorption and electrochemical behaviour of LAPd molecules onto HOPG (Fig. 3A and B).

AFM images of LAPd electrodeposited on HOPG at \( E_{\text{dep}} = -1.00 \) V showed small nanoparticles, with irregular shape and dimensions of approximately 3–7 nm height, randomly dispersed into a complex, 1.4 ± 0.2 nm height, two-dimensional network spread over the HOPG terraces (Fig. 3A).

AFM studies performed in palladium(II) containing solutions demonstrated that at \( E_{\text{dep}} = -1.00 \) V, Pd(0) nanoparticles and nanowires were electrodeposited on the surface of HOPG [23]. Therefore, the application of a high negative potential caused the dissociation of the LAPd complex and Pd(0) deposition as nanoparticles, that were embedded into the observed network film formed by a mixture of LA and LAPd molecules (Fig. 3A). The network has many pores, the dark regions in the image, leading to exposed HOPG surface at the bottom of the pores and presents a minor surface coverage when compared with the LAPd multilayer films.
spontaneously adsorbed onto HOPG from the same solution concentration.

AFM images of LAPd electrodeposited at $E_{\text{dep}} = +1.2 \, \text{V}$ showed a two-dimensional film (Fig. 3B) with a very compact morphology formed by small densely packed globular aggregates. The film presents both narrow pores as well as large ones that revealed uncovered HOPG, allowing the measurement of the film thickness of $2.7 \pm 0.3 \, \text{nm}$. The application of a positive potential induced the oxidation of the LAPd complex and the formation of a mixed multilayer of palladium oxides, LA and LAPd onto HOPG.

3.1.1.4. Spontaneous adsorption of Poly-MVATM onto HOPG. The Poly-MVATM modified HOPG was achieved by spontaneous adsorption during 3 min as described in Procedure 1. AFM images of adsorption from $3.3 \, \mu\text{g mL}^{-1}$ Poly-MVATM (containing $10 \, \mu\text{M LAPd}$) showed only a few molecules on the HOPG surface, assembled as small spherical $1.2 \pm 0.3 \, \text{nm}$ height aggregates (Fig. 4A). For higher concentrations of $33 \, \mu\text{g mL}^{-1}$ Poly-MVATM (containing $100 \, \mu\text{M LAPd}$), a uniform but incomplete film was observed, with the molecules forming thick globular filaments of $1.6 \pm 0.4 \, \text{nm}$ height (Fig. 4B).

3.1.1.5. Comparison between LA, LAPd and Poly-MVATM spontaneous adsorption. The topography of the LAPd modified HOPG suggests that LAPd adsorbs strongly onto HOPG surface even at a very low concentration of $0.1 \, \mu\text{M LAPd}$ (Fig. 2A), when compared with LA molecules that only form incomplete monolayer films at $400 \, \mu\text{M}$
LA (Fig. 1A). The LAPd greater adsorption onto HOPG is due to the presence of palladium into the LA structure (Scheme 1), which facilitates the interaction with the HOPG substrate. Indeed, complementary AFM studies showed that palladium(II) presents spontaneous adsorption onto the HOPG electrode from either PdSO4 or PdCl2 solutions.

In the case of Poly-MVA™ modified HOPG, and for the corresponding concentration of LAPd in solution (10 μM or 100 μM LAPd), a decreased surface coverage was observed (Fig. 4A and B), in comparison with the LAPd modified HOPG (Fig. 2C and D). The Poly-MVA™ active ingredient is a LAPd polymer that exists as a trimer of LAPd joined to thiamine [1,2]. Consequently, in Poly-MVA™, palladium is more hidden into the complex polymeric structure, decreasing the possibility of direct LAPd interaction with the carbon electrode surface, which explains the lower adsorption of the Poly-MVA™.

3.1.2. Voltammetric characterization of LAPd and Poly-MVA™

3.1.2.1. Redox behaviour of LA, LAPd and Poly-MVA™. The redox behaviour of LA was investigated by CV and DPV at GCE in 100 μM LA in 0.1 M phosphate buffer pH 7.0, and one anodic irreversible peak occurred at $E_{pa} = +0.80$ V (Fig. 1E and F) that was identified as the α-lipoic acid oxidation to β-lipoic acid [18].

Next, the voltammetric behaviour of LAPd at GCE was investigated by CV in 1 mM LAPd in 0.1 M phosphate buffer pH 7.0, $N_2$ saturated solution (Fig. 2) starting scanning at $E_i = 0.00$ V, in the positive direction, between the potential limits of $E_1 = +1.40$ V and $E_2 = −1.00$ V. Upon extensive cycling in the solution, an anodic peak at $E_{pa} = +1.20$ V and a cathodic peak at $E_{pc} = −0.40$ V (Fig. 2E) appeared. After 10 CV scans, the GCE was rinsed and transferred to 0.1 M acetate buffer pH 4.5 only. The voltammogram showed clearly a more complex redox behaviour at this pH (Fig. 2F) in agreement with the electrochemical behaviour of electrodeposited palladium nanostructures onto GCE [23].

The voltammetric behaviour of Poly-MVA™ at GCE was studied by CV in 1.3 mg mL$^{-1}$ of Poly-MVA™ (containing 4 mM LAPd) in 0.1 M phosphate buffer pH 7.0. The CV experiments showed three oxidation peaks in the first scan (data not shown), which did not increase upon continuous cycling (10 CV scans) in the same solution. When the GCE was transferred to 0.1 M acetate buffer pH 4.5, the voltammogram showed in the first CV scan only one oxidation peak. Using DPV, scanning from $E_i = 0.00$ V to $E_1 = +1.40$ V, a better assessment of the oxidation processes of Poly-MVA™ and the comparison with the LAPd oxidation results (Fig. 5) was possible.

The DPV recorded in 1 mM LAPd in 0.1 M phosphate buffer pH 7.0 solution showed a well defined oxidation peak, $E_{pa} = +1.11$ V, while in the case of 1.3 mg mL$^{-1}$ Poly-MVA™ (4 mM LAPd) in 0.1 M phosphate buffer pH 7.0 solution, similarly to the CVs three oxidation peaks occurred at $E_{pa} = +0.57$ V, +0.85 V and +1.30 V (Fig. 5A).

After 3 min spontaneous adsorption directly from the stock solutions of LAPd (10 mM), Poly-MVA™ (13 mg mL$^{-1}$, containing 40 mM LAPd), and 1 mM PdCl2 on GCE surface, the electrode was
transferred to 0.1 M acetate buffer pH 4.5 and DPVs (Fig. 5B) were recorded. The palladium characteristic voltammetric behaviour [23], seen in the voltammograms of Fig. 2E, was also observed after transferring the GCE to buffer (Figs. 2F and 5B). The voltammetry of the LAPd complex at the GCE and the AFM results are in agreement with the proposed LAPd structure (Scheme 1) that palladium incorporated into LA facilitates the interaction with the carbon substrates. The results obtained by DPV after spontaneous adsorption from either PdCl₂ or the LAPd solution (Fig. 5B) show the characteristic peak for palladium oxide formation during the voltammetric scan, \( E_{pa} = +1.15\) V in 0.1 M acetate buffer pH 4.5.

In the case of Poly-MVA\textsuperscript{TM}, the results showed that while three successive oxidation processes occur when the voltammogram was recorded in the Poly-MVA\textsuperscript{TM} containing solution (Fig. 5A), when the GCE was transferred to buffer an oxidation peak occurring at \( E_{pa} = +1.10\) V appeared, which is not related with the palladium oxide formation. The anodic peak at \( E_{pa} = +0.85\) V corresponds to the free LA present in Poly-MVA\textsuperscript{TM}, confirmed by standard addition of LA [18]. The differences in the oxidation potential between the standard LA (Fig. 1E and F) and LA in Poly-MVA\textsuperscript{TM} (Fig. 5A) are small and due to a matrix effect. The oxidation processes at \( E_{pa} = +0.57\) V is related to the oxidation
of other components present in the complex matrix of the Poly-MVA\textsuperscript{TM}.

3.1.2.2. Electrodeposition of LAPd and Poly-MVA\textsuperscript{TM}. Electrodeposition of LAPd and Poly-MVA\textsuperscript{TM} on GCE was carried out by applying the potentials $E_{\text{dep}} = -1.0\,\text{V}$ or $E_{\text{dep}} = +1.2\,\text{V}$, during 30 min, Procedure 3, in 1 mM LAPd or 1.3 mg mL\textsuperscript{-1} Poly-MVA\textsuperscript{TM} (containing 4 mM LAPd) in 0.1 M phosphate buffer pH 7.0.

A very complex redox behaviour, with various charge transfer reactions occurring at positive and negative potentials was observed in the CVs recorded in buffer (Fig. 3C) after electrodeposition at $E_{\text{dep}} = -1.0\,\text{V}$ in a 1 mM LAPd solution. The shape of the voltammetric wave changed upon cycling, in the subsequent scans a large anodic peak at $E_{\text{pa}} = +0.06\,\text{V}$ and, after reversing the scan direction, a sharp cathodic peak at $E_{\text{pc}} = +0.05\,\text{V}$, were found. After electrodeposition at $E_{\text{dep}} = +1.20\,\text{V}$ from 1 mM LAPd solution, the CVs obtained in 0.1 M acetate buffer pH 4.5 showed only small reduction peaks, at $E_{\text{pc}} = +0.06\,\text{V}$ and $E_{\text{pa}} = +0.05\,\text{V}$, which improved slightly in the subsequent scans (Fig. 3D).

Voltagometric studies performed in palladium containing solutions [23] showed that palladium is electrodeposited on GCE as Pd(0), after applying a negative potential, or palladium oxides, after applying a positive potential. Applying the potential of $E_{\text{dep}} = -1.0\,\text{V}$ ensured that more palladium could be removed from the LAPd complex and deposited at GCE as Pd(0), than in the case of continuous cycling (Fig. 5A and B) the voltammograms showing the characteristic palladium electrochemical behaviour [23], i.e. oxide formation and their reduction at positive potentials and hydrogen incorporation at negative potentials (Fig. 3C). Electrodeposition at $E_{\text{dep}} = +1.20\,\text{V}$ was also performed in order to confirm that palladium could be removed from the LAPd complex and deposited as palladium oxides. The peaks obtained in buffer only, although smaller than in the case of $E_{\text{dep}} = -1.0\,\text{V}$ electrodeposition, improved in the subsequent recorded scans, corresponding to reduction of the palladium oxides deposited on GCE to Pd(0) (Fig. 3D).

Whereas after the electrodeposition performed in the solution of 1.3 mg mL\textsuperscript{-1} Poly-MVA\textsuperscript{TM} at $E_{\text{dep}} = -1.00\,\text{V}$, the CVs showed in buffer an oxidation peak, $E_{\text{pa}} = +1.37\,\text{V}$ (Fig. 4C) after electrodeposition at $E_{\text{dep}} = +1.20\,\text{V}$ from the same Poly-MVA\textsuperscript{TM} solution no peaks were observed (Fig. 4D). This means that the voltammetric behaviour of Poly-MVA\textsuperscript{TM} (Fig. 6) cannot be associated with the known electrochemistry of palladium (Fig. 3C and D), although a charge transfer reaction was observed in the first voltammogram, no other peaks could be detected in the following scans.

3.2. Interaction of dsDNA with LAPd and Poly-MVA\textsuperscript{TM}

3.2.1. AFM characterization of dsDNA–LAPd and dsDNA–Poly-MVA\textsuperscript{TM} interaction

The mechanism of interaction of LAPd and Poly-MVA\textsuperscript{TM} with dsDNA was investigated and characterized by AFM. The films on HOPG were prepared depositing layer by layer dsDNA–LAPd (Procedure 4) and LAPd–dsDNA (Procedure 5), and from incubated solutions of dsDNA–LAPd and dsDNA–Poly-MVA\textsuperscript{TM} (Procedure 6).

In order to have the dsDNA control adsorption pattern, AFM was employed to study spontaneous adsorption of dsDNA from solutions of 5 μg mL\textsuperscript{-1} and 10 μg mL\textsuperscript{-1} dsDNA, as described in Procedure 1. The AFM topographical images in air of the dsDNA film obtained from 5 μg mL\textsuperscript{-1} dsDNA showed coiled dsDNA molecules of 1.5 ± 0.6 nm height (Fig. 6A), while from 10 μg mL\textsuperscript{-1} dsDNA showed a thin network of 1.1 ± 0.2 nm height (Fig. 4E).

The layer by layer dsDNA–LAPd modified HOPG, Procedure 4 (Fig. 6B), was obtained by LAPd spontaneous adsorption from a solution of 5 μM LAPd onto the control dsDNA modified HOPG obtained from 5 μg mL\textsuperscript{-1} dsDNA (Fig. 6A). The AFM images showed small aggregates of molecules, as well as looped filaments with portions of linear dsDNA. The measured thickness of the dsDNA–LAPd film was of 1.3 ± 0.4 nm.

The layer by layer LAPd–dsDNA modified HOPG surfaces, Procedure 5 (Fig. 6C and D), were obtained by dsDNA spontaneous adsorption from a solution of 5 μg mL\textsuperscript{-1} dsDNA onto a LAPd modified HOPG prepared from LAPd solutions of 0.1 μM (Fig. 2A) or 1 μM (Fig. 2B). The AFM images of the LAPd–dsDNA modified HOPG (0.1 μM LAPd) (Fig. 6C) showed a loosely packed film of 1.6 ± 0.3 nm height and spherical aggregates, up to 5 nm height, adsorbed on the HOPG areas uncovered by the LAPd–dsDNA. The AFM images of the LAPd–dsDNA modified HOPG (1 μM LAPd) (Fig. 6D) showed a non-compact, granular film of 1.4 ± 0.2 nm height, and unknotted extended dsDNA molecules could be imaged inside the LAPd–dsDNA film. The measured thickness of the layer by layer dsDNA–LAPd and LAPd–dsDNA films presented heights consistent with both the height of the coiled dsDNA molecules and LAPd films, leading to inconclusive results concerning the LAPd
interaction with dsDNA. Therefore the LAPd interaction with dsDNA in solution was also AFM morphologically evaluated.

The HOPG electrode was modified by a dsDNA–LAPd film obtained by spontaneous adsorption during 3 min from incubated solutions of 5 \( \mu \text{g mL}^{-1} \) dsDNA with 0.1 \( \mu \text{M} \) or 5 \( \mu \text{M} \) LAPd, as described in the Procedure 6. AFM images of dsDNA–LAPd modified HOPG, from 0.1 \( \mu \text{M} \) LAPd, showed the HOPG surface covered by small aggregates, a few twisted molecules, as well as a high number of unknotted extended dsDNA molecules of 1.2 ± 0.2 nm height (Fig. 6E). Using 5 \( \mu \text{M} \) LAPd, the AFM images showed the formation of a network with thick filaments of 1.7 ± 0.2 nm height, and a number of dsDNA molecules arranged near the step edges of the HOPG were also observed inside the network film (Fig. 6F).

Fig. 6. AFM images of: (A) control dsDNA modified HOPG (Procedure 1) from 5 \( \mu \text{g mL}^{-1} \) dsDNA, (B) dsDNA–LAPd modified HOPG (Procedure 4), (C and D) LAPd–dsDNA modified HOPG (Procedure 5): (C) 0.1 \( \mu \text{M} \) and (D) 1.0 \( \mu \text{M} \) LAPd, (E and F) dsDNA–LAPd modified HOPG (Procedure 6): (E) 0.1 \( \mu \text{M} \) and (F) 5.0 \( \mu \text{M} \) LAPd. See Section 2 for details.

In conclusion, in the presence of lower concentration of LAPd the dsDNA molecules appeared less tangled and bended, and more extended on the HOPG surface (Fig. 6B–F), when compared with the dsDNA molecules adsorbed from the control dsDNA solution of the same concentration (Fig. 6A).

For the AFM morphological evaluation of the Poly-MVA\textsuperscript{TM} interaction with dsDNA in solution, the HOPG electrode was modified by a dsDNA–Poly–MVA\textsuperscript{TM} film obtained by spontaneous adsorption during 3 min from incubated solutions of 10 \( \mu \text{g mL}^{-1} \) dsDNA with 3.3 \( \mu \text{g mL}^{-1} \) Poly-MVA\textsuperscript{TM} (containing 10 \( \mu \text{M} \) LAPd), as described in the Procedure 6. AFM images showed a layer formed by tight loops and areas of the HOPG that were not covered at all (Fig. 4F). The dsDNA–Poly-MVA\textsuperscript{TM} film was composed by aggregates of
molecules of 1.8 ± 0.3 nm height, larger than the expected height of the dsDNA, which suggest a complex interaction of the dsDNA with the various molecules existent in the Poly-MVATM solution.

### 3.2.2. Voltammetric characterization of dsDNA–LAPd and dsDNA–Poly-MVATM interaction

The dsDNA interaction with LAPd and Poly-MVATM was studied in order to investigate the palladium complex action in inducing double helix deconformation, hydrogen bonding cleavage and/or oxidative damage to DNA bases. The interaction was followed by DPV, and the observed changes of the purine bases' oxidation peak currents, desoxyguanosine (dGuo), $E_{pa} = +1.03$ V, and desoxyadenosine (dAdo), $E_{pa} = +1.30$ V, were compared with the results obtained for a dsDNA control solution. The occurrence of the guanine or/and adenine oxidation product peaks, the biomarkers 8-oxyguanine and 2,8-dihydroxyadenine, at $E_{pa} \sim +0.45$ V in 0.1 M acetate buffer pH 4.5, is an indication of oxidative damage caused to DNA [24,25].

The GCE was modified with a thin-layer of dsDNA, Procedure 7, and then incubated for different periods of time with either 1 mM LAPd or 1.3 mg ml$^{-1}$ Poly-MVATM [4 mM LAPd] in 0.1 M phosphate buffer pH 7.0. The DPV recorded in 0.1 M acetate buffer pH 4.5 showed changes in the peak currents corresponding to the oxidation of dGuo and dAdo, respectively, when compared with the results obtained for the dsDNA control solution (Fig. 7). However, other oxidation processes were also detected, the peak at $E_{pa} = +1.18$ V in the case of dsDNA–LAPd incubation (Fig. 7A) and oxidation peaks at $E_{pa} = +0.55$ V, $E_{pa} = +0.80$ V and $E_{pa} = +1.27$ V in the case of dsDNA–Poly-MVATM incubation (Fig. 7B) and all increased with increasing incubation times.

In another experiment, the interaction of LAPd with dsDNA was studied at GCE after 48 h incubation time, Procedure 8. The DPVs recorded in acetate buffer showed very small dGuo and dAdo peak currents, compared with the dsDNA control solution, and a large oxidation peak which rapidly increases in the following DPV scans, $E_{pa} = +1.18$ V, corresponding to the joint deposition of palladium oxides that hinders the DNA oxidation processes (Fig. 7C). The voltammetric results led to the conclusion that modification of the dsDNA structure occurs upon interaction with LAPd, recognized through the decrease of the dGuo and dAdo oxidation peaks (Fig. 7A and C). Moreover, no oxidative damage to dsDNA was detected, as no oxidation peaks corresponding to DNA oxidative damage were noted in the DPVs recorded either after short (10 min) or long (48 h) incubation times.

The interaction of dsDNA with Poly-MVATM (Fig. 7B) was found to be difficult to assess when compared to the interaction of dsDNA with LAPd, due to the other redox active compounds existent in the Poly-MVATM matrix. However, as no oxidation peaks of 8-oxyguanine or 2,8-dihydroxyadenine were observed in the DPV scans, it was concluded that there was no oxidative damage caused to dsDNA by Poly-MVATM.

### 4. Conclusions

The interaction of dsDNA with LAPd and Poly-MVATM was studied at room temperature, using AFM and voltammetry, showing that the interaction with either caused no oxidative damage to dsDNA. The interaction of dsDNA with low concentrations of the LAPd complex lead to less knotted and bended, and more extended dsDNA molecules on the HOPG surface, when compared with control dsDNA adsorbed on the HOPG.

The adsorption process and the redox behaviour of LA, LAPd and Poly-MVATM presents characteristic adsorption patterns and degree of HOPG surface coverage, depending on the chemical structures, the dimensions, the solution concentration and the applied potential. The LAPd molecules interact and adsorb strongly on HOPG, in comparison with LA, probably due to the incorporation of palladium into the ligand structure.

By applying high negative or high positive potentials, the stability of both the LAPd complex and the LAPd-containing Poly-MVATM solution was tested. The voltammetric and AFM results show that while in the case of LAPd complex palladium removal is still possible, in the Poly-MVATM solution the same is not achieved,
most likely due to palladium being sequestered within the LAPd polymer joined to thiamine.

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