1. Introduction

Epidemiological studies have shown that occupational and environmental exposure to some metals is associated with increased risk of various cancers and adverse health effects [1]. Metals are found throughout the environment, and although certain metals are highly toxic, others are essential to living systems, being required for enzymatic reactions or other physiological processes. The basis of the interaction of toxic metals such as Pb, Cd, Ni and Pd with cellular constituents involves their coordination with biological ligands, and the non-specific transport of these toxic metals interferes with the transport of essential metals.

Concerning metal–DNA interactions, transition and post-transition metal ions can interact specifically with double-stranded DNA (dsDNA), usually at the negatively charged phosphate groups or at the nitrogenous bases that are generally approached through the minor and major grooves [2,3]. Due to the interaction with the acceptor sites of the purine and pyrimidine bases [4], especially the N7 of guanine and adenine, the N3 of cytidine and the carbonyl oxygen [2,5], these metal ions can induce partial disordering of the B-form DNA and reduction of base stacking and base pairing. Also, in the presence of transition metals, which have a higher affinity for DNA bases, aggregation of high-molecular-weight DNA has been observed [3–6].

The interaction of DNA with Pb2+, probably due to differences in experimental protocols, is not consensual [7–14]. For experimental conditions using Pb2+/DNA ratios of 1:1, Pb2+ has shown small interaction with dsDNA with little sign of deviation from the B-DNA conformation [11], due to the Pb2+ ions interacting mainly with the charged phosphate groups and only weakly with the nucleic bases. For higher Pb2+/DNA concentration ratios, Pb2+ causes destabilization of the DNA double-helix [12,13], binds to the N7 position of guanine, and also interacts with the A–T base pairs.

Both Cd2+ and Ni2+ interact extensively with DNA in acceptor sites on the N7 position of purines and N3 position of pyrimidines and destabilize the double helix in concentrated solutions of high-molecular-weight DNA at room temperature [4–6]. Crystallographic, infrared and Raman spectroscopy studies demonstrated the binding of Cd2+ to the N7 position of guanine, A–T regions, N3 position of pyrimidines, phosphates, and also to sugar hydroxyls, contrary to observations with other transition and post-transition metals [2,5,6,15–17]. It has been reported that Ni2+ also binds to the N7 position of guanine and this is stabilized by chelation of the phosphate group [4,6,18].

The interaction of Pd2+ with DNA molecules can induce conformational changes in DNA structures [19]. More recent results show that palladium induces nucleoside conformational changes coupled with binding to N7, and possibly also to N1, the gua-

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**Electrochemical and AFM evaluation of hazard compounds–DNA interaction**

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**A R T I C L E   I N F O**

Article history:
Received 28 April 2008
Received in revised form 13 June 2008
Accepted 11 July 2008
Available online 23 July 2008

Keywords:
Lead
Cadmium
Nickel and palladium metal cations
DNA
Metal–DNA complex
AFM
Differential pulse voltammetry
Oxidative damage

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

The evaluation of the interaction of hazard compounds, divalent cations Pb, Cd, Ni and Pd, with double-stranded DNA (dsDNA), forming a metal–DNA complex, was studied by atomic force microscopy (AFM) and differential pulse voltammetry (DPV) on two carbon electrode surfaces, highly oriented pyrolytic graphite (HOPG) and glassy carbon (GC). The electrochemical behaviour of these metal–DNA complexes was related to the different adsorption patterns and conformational changes obtained by the AFM images on the HOPG surface. The dsDNA interaction was specific with each metal cation, inducing structural changes in the B-DNA structure, local denaturation of the double helix and oxidative damage. AFM images showed an increase of the electrode surface coverage for lead, cadmium and nickel DNA complexes. For cadmium and nickel–DNA complexes oxidative damage to DNA was electrochemically detected for the concentrations studied. Palladium interaction with dsDNA induced condensation of the dsDNA secondary structure, which led to the aggregation of helixes forming very compact and thick filaments in the network film. The voltammetric data for the palladium–DNA complex showed a sharp decrease of the guanosine and adenosine oxidation peak currents, consistent with the AFM results of DNA condensation in the presence of palladium, but no DNA oxidative damage was detected for the range of concentrations used.

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nine acceptor site. In addition, significant perturbation of the thymine ring and the phosphodioxynucleotide DNA groups was described, together with complete denaturation of the B-DNA secondary structure [4].

However, consensus over the transition and post-transition metal ions’ direct interaction with dsDNA is still needed. In the present paper, a systematic study to elucidate the mechanism of interaction of the Pb(II), Cd(II), Ni(II) and Pd(II) with dsDNA, which leads to the formation of the corresponding metal–DNA complexes, was carried out on two different carbon electrodes, highly oriented pyrolytic graphite (HOPG) and glassy carbon (GC) using magnetic AC mode atomic force microscopy (MAC Mode AFM) and voltammetry. AFM can give important information concerning DNA structural modifications with extraordinary resolution and accuracy, while electrochemical techniques are successfully employed for the rapid detection of small perturbations of the double helical structure, due to their high sensitivity and selectivity, when compared with other methods reported in the literature. The results obtained revealed important correlations between the different adsorption patterns due to the conformational changes imaged for each metal–DNA complex obtained on the HOPG surface and their voltammetric behaviour on GC electrodes.

2. Experimental

2.1. Materials and reagents

Calf thymus dsDNA, polyguanylic (poly[G]) and polyadenylic (poly[A]) acids (from Sigma–Aldrich), lead(II) nitrate, cadmium(II) nitrate, nickel(II) chloride and potassium tetrachloropalladate(II) (from Merck) were used without further purification. All solutions were prepared using analytical grade reagents and purified water (from Millipore Milli-Q system (conductivity ≤ 0.1 µS cm⁻¹)).

Stock solutions of 2 mM Pb(II), Cd(II), Ni(II) and Pd(II) were prepared directly in pH 4.5 0.2 M acetate buffer supporting electrolyte. A stock solution of 300 µg mL⁻¹ dsDNA was prepared in deionised water and kept at 4 °C. The solutions were diluted to the desired concentration by mixing buffer supporting electrolyte.

Microvolumes were measured using EP-10 and EP-100 Plus Motorized Microliter Pippettes (Rainin Instrument Co. Inc., Woburn, USA). The pH measurements were carried out using a Crison micropH 2001 pH-meter with an Ingold combined glass electrode. All experiments were done at room temperature (25 ± 1 °C).

2.2. Atomic force microscopy

HOPG, grade ZYB of 15 mm × 15 mm × 2 mm dimensions, from Advanced Ceramics Co., was used as a substrate in the MAC Mode AFM study. The HOPG was freshly cleaved with adhesive tape prior to each experiment and imaged by 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 Molecular Imaging Corp., Tempe, AZ. All the AFM experiments were performed with a CS AFM S scanner with a scan range 6 µm in x – y and 2 µm in z, from Molecular Imaging Corporation. Silicon type II MAClevers of 225 µm length, 2.8 N m⁻¹ spring constants and 60–90 kHz resonant frequencies in air (Molecular Imaging Co.) were used. All the AFM topographical images (256 samples/line × 256 lines) were taken at room temperature; scan rates 0.8–2.0 lines s⁻¹. When necessary, MAC Mode 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, Molecular Imaging Co and with Origin version 6.0 from Microcal Software, Inc., USA.

2.3. Voltammetric parameters and electrochemical cells

Voltammetric experiments were carried out using a µAutolab running with GPES 4.9 software, Eco-Chemie, Utrecht, The Netherlands. The experimental conditions for differential pulse (DP) voltammetry were pulse amplitude 50 mV, pulse width 70 ms and scan rate 5 mV s⁻¹. Measurements were carried out 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, in a 0.5 mL one-compartment electrochemical cell.

The GCE was polished using diamond spray (particle size 1 µm) before every electrochemical assay. After polishing, the electrode was rinsed thoroughly with Milli-Q water for 30 s; then it was sonicated for 1 min in an ultrasonic bath and again rinsed with water. After this mechanical treatment, the GCE was placed supporting electrolyte and various DP voltammograms were recorded until a steady state baseline voltammogram was obtained. This procedure ensured very reproducible experimental results.

All the DP voltammograms presented were background-subtracted and baseline-corrected using the moving average application with a step window of 5 mV included in GPES version 4.9 software. This mathematical treatment improves the visualization and identification of peaks over the baseline without introducing artefacts, although the peak current intensity is in some cases reduced (<10%) relative to that of the untreated curve. The mathematical treatment of the original voltammograms was used in the presentation of all experimental voltammograms for a better and clearer identification of the peaks.

2.4. Samples preparation for atomic force microscopy and voltammetric studies

Metal–DNA, metal–poly[G] and metal–poly[A] complexes were prepared by incubation of dsDNA, poly[G] and poly[A] in pH 4.5 0.2 M acetate buffer with different concentrations of the desired metal ion solution. Control solutions of dsDNA, poly[G] and poly[A] in pH 4.5 0.2 M acetate buffer were also prepared and stored during the same periods of time in similar conditions as the metal–DNA incubated solutions.

For the AFM experiments, each metal–DNA complex was prepared incubating 100 µM of the metal ion solution with 10 µg mL⁻¹ dsDNA in pH 4.5 0.2 M acetate buffer at room temperature, during 24 h. Two hundred microliter samples of either the dsDNA control solution or the metal–DNA complex solution were placed onto the freshly cleaved HOPG surface for 10 min. The excess of solution was gently cleaned with a jet of Millipore Milli-Q water, and the HOPG with adsorbed dsDNA or metal–DNA complex was then dried in a sterile atmosphere and imaged by MAC Mode AFM in air.

For the voltammetric study, 60 µg mL⁻¹ dsDNA, 10 µg mL⁻¹ poly[G] or poly[A] in pH 4.5 0.2 M acetate buffer, were incubated at room temperature with different concentrations of the metal ion solution, during different periods of time: 6, 12, 24 and 60 h. The immobilisation on the surface of the GCE of the thin-layer films of dsDNA, poly[G] and poly[A] from control solutions and of the thin-layer films of the metal–DNA, metal–poly[G] and metal–poly[A] complexes, and Pb(II) was by electroadsorption at +0.30 V vs. Ag/AgCl (3 M KCl), during 300 s.

The results obtained revealed important correlations between the different adsorption patterns due to the conformational Changes imaged for each metal–DNA complex obtained on the HOPG surface and their voltammetric behaviour on GC electrodes.
3. Results

3.1. Atomic force microscopy evaluation of metal–DNA interactions

In order to understand the dsDNA conformational modifications after interaction with the metal ions Pb²⁺, Cd²⁺, Ni²⁺ and Pd²⁺, the spontaneous adsorption of each metal–DNA complex on a HOPG electrode surface was investigated using AFM in air. The complexes were prepared by incubation during 24 h of 10 μg mL⁻¹ dsDNA with 100 μM Cd²⁺, Ni²⁺, Pb²⁺ or Pd²⁺, as described in Section 2.4.

An atomically smooth HOPG electrode, with less than 0.06 nm root-mean-square (r.m.s.) roughness for a 1000 nm × 1000 nm surface area, was used in the AFM study, in order to clearly resolve the morphological characteristics of each metal–DNA complex. The GCE used for the electrochemical characterization has a rough surface with 2.10 nm r.m.s. roughness for the same surface area, therefore unsuitable for AFM surface characterization [20].

First, AFM was employed to study the dsDNA spontaneous adsorption from a control solution of 10 μg mL⁻¹ dsDNA stored during 24 h (Figs. 1A, 2A, 3A and 4A). The dsDNA molecules adsorbed spontaneously onto HOPG and self-organized in a non-compact thin network, with a thickness of 2.0 ± 0.5 nm, Fig. 1D, leading to exposed HOPG surface at the bottom of the holes.

AFM images of Pb–DNA complexes adsorbed onto HOPG, Fig. 1B, showed a uniform thin film network, forming looped filaments with portions of uncovered HOPG surface (Fig. 1B). The measured thickness of the Pb–DNA film presented heights of 1.0 ± 0.2 nm, as observed in the section analysis graph (Fig. 1E). AFM images of Cd–DNA complexes adsorbed onto HOPG, Fig. 2B, showed the surface almost completely covered and presenting only a few pores, the dark regions in the images, leading to a very little uncovered HOPG surface. The average height and standard deviation of the Cd–DNA film was 1.2 ± 0.3 nm (Fig. 2D). AFM images of Ni–DNA complexes adsorbed onto the HOPG, Fig. 3B, also showed the surface to be almost completely covered and with very few pores. The average height and standard deviation of the Ni–DNA film was 1.3 ± 0.4 nm (Fig. 3D).

AFM images of Pd–DNA complexes adsorbed onto the HOPG, Fig. 4B and C, showed the molecules self-assembled in a very thick two-dimensional lattice, formed by thick and coiled filaments which joined together in end-to-end aggregation on the HOPG surface. The Pd–DNA aggregates were twisted together in large and rather flexible loops, leaving big parts of the HOPG surface uncovered. The measured thickness along the network filaments was highly irregular, with heights varying between 2.0 and 13.0 nm (Fig. 4D and E).

Fig. 1. AFM images: (A) dsDNA and (B) Pb–DNA complex. (C) DP voltammograms in buffer of an immobilised thin-layer film on GCE of: (—) dsDNA from control solution after 24 h preparation, (—•••) Pb–DNA complex from a solution incubated with 1 mM Pb²⁺ during 24 h, and (•••••) electroadsorbed Pb²⁺ ions from a solution of 1 mM Pb²⁺. (D and E) Cross-section profiles through white lines in the images A and B. See Section 2.4 for experimental details.
3.2. Voltammetric evaluation of metal–DNA interactions

The ability of Pb\(^{2+}\), Cd\(^{2+}\), Ni\(^{2+}\) and Pd\(^{2+}\) to induce hydrogen bonding cleavage, double helix deconformation and/or oxidative damage to DNA bases was studied using DP voltammetry. In all experiments, different concentrations of the metal ions were incubated for different periods of time with dsDNA and their interaction was evaluated using an immobilised thin-layer film of metal–DNA complex electrodeposited at +0.30 V during 300 s, onto the GCE surface from the incubated solutions, as described in Section 2.4.

The observed changes in the dsDNA oxidation peak currents of the purine bases, deoxyguanosine (dGuo) at \(E_{pa} = +1.03\) V, and deoxyadenosine (dAdo) at \(E_{pa} = +1.30\) V, were compared with the results obtained for a dsDNA control solution. The occurrence of the guanine or/and adenine oxidation product peaks, 8-oxoguanine and 2,8-dihydroxyadenine, at \(E_{p} \sim +0.45\) V vs. Ag/AgCl, in pH 4.5 0.2 M acetate buffer, is an indicator of oxidative damage caused to DNA [21,22].

The electroadsorption potential, +0.30 V vs. Ag/AgCl, used for the preparation of immobilised thin-layer film of the metal–DNA complexes was selected to strengthen the adsorption of DNA on the GCE surface, but it is a positive potential lower than the oxidation potential of any DNA base, or base oxidation product [21,22].

The thin-layer Pb–DNA complex was prepared from incubated solution of dsDNA with 1 mM Pb\(^{2+}\) during 24 h. The electrode was then transferred to the supporting electrolyte solution where DP voltammetry was performed. The voltammogram obtained showed an increase of the oxidation peak of dGuo relative to the voltammogram recorded for the dsDNA control solution, Fig. 1C, as well as a new peak at \(E_{pa} = +1.33\) V. In order to clarify the occurrence of this peak, electroadsorption at +0.30 V was carried out during 300 s from a buffer solution containing only 1 mM Pb\(^{2+}\), in the absence of dsDNA. The DP voltammogram recorded in buffer, Fig. 1C, showed only one peak at \(E_{pa} = +1.33\) V and confirmed that it corresponds to the formation of lead oxide on the GCE surface in a process involving only the Pb\(^{2+}\) cations.

The interaction between 2 mM Cd\(^{2+}\) ions and dsDNA was initially studied after 6 h of incubation. The thin-layer Cd–DNA complex was prepared from the thin-layer film Cd–DNA complex in buffer showed an increase of the oxidation peaks of dGuo and dAdo after 24 h incubation the DP voltammogram obtained showed a small decrease in the oxidation peaks of dGuo and dAdo, compared with 6 h of incubation, and a new peak appeared at \(E_{pa} = +0.45\) V (Fig. 2C).
The thin-layer film of Ni–DNA complex was prepared from a solution of dsDNA incubated with 2 mM Ni²⁺ during 12 and 24 h. The DP voltammograms recorded in supporting electrolyte showed a gradual increase of the oxidation peaks of dGuo and dAdo, in a time-dependent manner (Fig. 3C). Again, a new peak, at \( E_{pa} = +0.45 \text{ V} \), on the voltammogram recorded after 24 h incubation was observed.

Similar experiments were carried out for different incubation times and concentrations of Pb²⁺, Ni²⁺, and Cd²⁺ (not shown). The increase in current of the oxidation peaks of dGuo and dAdo and of the peak at \( E_{pa} = +0.45 \text{ V} \) was proportional to the increase of metal ion concentration and incubation time.

In order to identify the peak at \( E_{pa} = +0.45 \text{ V} \), several experiments were performed using Pb²⁺, Ni²⁺ and Cd²⁺ solutions incubated with polynucleotides of known sequences, namely poly[G] and poly[A] (not shown). The incubation and the deposition onto the GCE surface of metal–poly[G] and metal–poly[A] complexes was as described in Section 2.4. The DP voltammograms of the thin-layer films of metal–poly[G] or metal–poly[A] complexes obtained in buffer showed the variation in the peak current corresponding to the oxidation of dGuo or dAdo, respectively, and the small peak at \( E_{pa} = +0.45 \text{ V} \) appeared in both cases.

The Pd–DNA complex was prepared by incubating 60 μg mL⁻¹ dsDNA with solutions containing 10 μM and 100 μM Pd²⁺ (Fig. 5). First, the interaction of Pd²⁺ with DNA was studied directly in the incubated solutions (Fig. 5A and B). The DP voltammogram obtained for the Pd–DNA complex in solution after 24 h incubation of the dsDNA with 10 μM Pd²⁺ was an almost perfect overlay with the results obtained in the dsDNA control solution (Fig. 5A). However, when the DP voltammogram was recorded in solution after 60 h incubation in 10 μM Pd²⁺, a considerable decrease in the dGuo and dAdo oxidation peak currents occurred, when compared with the results obtained for the dsDNA control solution. The same experiment was performed using a more concentrated solution of 100 μM Pd²⁺ and the DNA oxidation peaks for dGuo or dAdo both disappeared in the DP voltammograms after 24 and 60 h incubation, Fig. 5B, suggesting a very strong interaction between Pd²⁺ and dsDNA. As no peaks were observed in the DP voltammograms, corresponding to the products of oxidation of dGuo or dAdo, 8-oxoguanine or 2,8-dihydroxiadenine, it was concluded that, for the experimental conditions used, there was no oxidative damage.

In another experiment, the thin-layer film of Pd–DNA complex was prepared from a solution of dsDNA incubated with 10 μM Pd²⁺ during 24 and 60 h (Fig. 5C). The DP voltammograms recorded in supporting electrolyte confirmed the trend of the previous experiments in the incubated solutions, Fig. 5A and B, i.e. a sharp decrease of the dGuo and dAdo oxidation peaks in the case of dsDNA – 10 μM Pd²⁺ incubated solutions, the absence of any peaks for dsDNA – 100 μM Pd²⁺ incubated solutions and no DNA oxidative damage.
4. Discussion

The AFM and voltammetric results obtained for the dsDNA interaction with Pb\textsuperscript{2+} (Fig. 1B and C), Cd\textsuperscript{2+} (Fig. 2B and C), Ni\textsuperscript{2+} (Fig. 3B and C) and Pd\textsuperscript{2+} (Figs. 4B, C and 5) have been evaluated and were all compared with the control dsDNA modified HOPG (Figs. 1A, 2A, 3A and 4A) or GCE (Figs. 1C, 2C, 3C and 5), subjected to the same treatment as the immobilised thin-layer films of dsDNA–metal complexes. The results were compared with the literature and were in agreement with a model proposed to describe the Pb\textsuperscript{2+}, Cd\textsuperscript{2+}, Ni\textsuperscript{2+} and Pd\textsuperscript{2+} interaction with dsDNA [4,5]. Metal cations interact with the dsDNA phosphate groups and bases, the purine N7 acceptors being the most probable metal-binding sites. Consequently, the DNA double-helix becomes locally denatured due to the unwinding of the double helix, displacement of bases relative to the backbone and disruption of the base pairing and base stacking. In the presence of certain divalent metal cations that exhibit higher affinity for DNA bases, especially purine N7 sites, the DNA strands partially opened can crosslink with neighbouring strands by metal ion bridges, leading to the formation of a partial DNA aggregated state [3–6].

AFM images showed a reorganization of the DNA self-assembled network on the HOPG surface following the interaction with each one of the four metal cations studied: Pb\textsuperscript{2+}, Cd\textsuperscript{2+}, Ni\textsuperscript{2+} and Pd\textsuperscript{2+}. An increase of electrode surface coverage and a decrease of network film thickness for the Pb–DNA, Cd–DNA and Ni–DNA complexes were observed, Figs. 1B, 1E, 2B, 2D, 3B and 3D, when compared with the control dsDNA film thickness, in the same experimental conditions, Figs. 1A, 1D, 2A and 3A, due to structural modification with local denaturation of the DNA double helix and single strand DNA formation. AFM images of Cd–DNA, Fig. 2B, and Ni–DNA, Fig. 3B, complexes presented a similar pattern, with a higher degree of surface coverage when compared with the Pb–DNA, Fig. 1B, confirming a stronger interaction of the Cd–DNA and Ni–DNA complexes with the HOPG. The bases initially protected inside the helix become more exposed to the solution and freer to undergo hydrophobic interactions with the hydrophobic carbon surface. The pattern of adsorption of these three metal–DNA complexes is consistent with coverage of the surface by single-stranded DNA molecules, which has an increased adsorption on carbon electrodes, compared with dsDNA [21].

The voltammetric results led to the conclusion that modification of the dsDNA structure occurs upon interaction with Pb\textsuperscript{2+}, Cd\textsuperscript{2+} and Ni\textsuperscript{2+}, and oxidative damage to DNA in the case of Cd\textsuperscript{2+} and Ni\textsuperscript{2+} was also detected.

The interaction between Pb\textsuperscript{2+} and DNA in the incubated solution is confirmed by the increase of the dGuo oxidation peak, Fig. 1C, but no oxidation product was detected. The free Pb\textsuperscript{2+} ions are oxidised at $E_{\text{pa}} = +1.33$ V, forming a lead oxide film on the GCE surface, Fig. 1C, which is electrodeposited with the Pb–DNA complex, hindering further detection of DNA damage. In the case of Cd\textsuperscript{2+}, Fig. 2C, and Ni\textsuperscript{2+}, Fig. 3C, the increase of the oxidation peaks for dGuo and dAdo
is a consequence of the close contact of the DNA bases with the GCE surface due to DNA conformational changes and unwinding of the double helix. The oxidative damage caused to DNA bases was confirmed by the occurrence of the peak at $E_{pa} = +0.45$ V (Figs. 2C and 3C). DNA probes of known sequences, e.g. poly[G] and poly[A], were used to check that Cd$^{2+}$ and Ni$^{2+}$ oxidative damage caused to DNA is not specific to one purinic base. In fact, the products of oxidation of the purinic bases, 8-oxoguanine and 2,8-dihydroxyadenine [21,22], peaks at $E_{pa} = +0.45$ V, were found due to the interaction of Cd$^{2+}$ and Ni$^{2+}$ with either poly[G] or poly[A].

The very compact Pd–DNA structures observed by AFM (Fig. 4) and the voltammetric results (Fig. 5) suggest that the Pd$^{2+}$ cations induced great morphological changes in the dsDNA structure. The thickness of the Pd–DNA complex lattice, Fig. 4D and E, presents values of 2.0–13.0 nm, higher than the thickness of the dsDNA control of 2.0 $\pm$ 0.47 nm, meaning that several DNA layers are involved in a condensation process. The voltammetric results, Fig. 5, showed that Pd$^{2+}$ interaction with dsDNA occurred at lower concentrations when compared with Pb$^{2+}$, Cd$^{2+}$ or Ni$^{2+}$. The peaks for both dGuo and dAdo residues decreased in 10 $\mu$M Pd$^{2+}$ incubated solutions, and no signals were detected when a higher concentration of 100 $\mu$M Pd$^{2+}$ was used. The strong Pd$^{2+}$ ion interaction with dsDNA is localized in the interior of the double helix, which locally denatures the B-form secondary structure, in agreement with a phenomenon previously observed in the presence of Pb$^{2+}$ by Raman spectroscopy [4]. The dsDNA local denaturation is rapidly followed by DNA aggregation, due to metal ion bridges formed between bases of the neighbouring strands. The strong and stable interaction between Pd$^{2+}$ and dsDNA is both time and concentration dependent, but causing no oxidative damage to DNA.

5. Conclusion

The AFM results and the voltammetric data have demonstrated that Pb$^{2+}$, Ni$^{2+}$, Cd$^{2+}$ and Pd$^{2+}$ interact specifically with the dsDNA, due to a high affinity of forming covalent bonds with nitrogenous bases, and inducing structural changes in B-DNA.

A reorganization of the DNA self-assembled network on the surface of the HOPG electrode was observed, resulting in an increase of the electrode surface coverage by the Pb–DNA, Cd–DNA and Ni–DNA complexes, when compared with the control dsDNA in the same experimental conditions. This phenomenon is related to a local denaturation of the double helix due to the metal–DNA interaction, facilitating the hydrophobic interactions between the DNA bases and the hydrophobic carbon electrode surface. Consistent with AFM results, the voltammetric data showed for all metals studied a metal–DNA interaction that lead to conformational modifications of the dsDNA double helix, as well as the occurrence of oxidative damage to DNA in the case of Cd$^{2+}$ and Ni$^{2+}$.

The Pd$^{2+}$ cations induced the greatest morphological changes in the DNA adsorption pattern, leading to the formation of thicker aggregates of the Pd–DNA complex caused by DNA condensation. The compact Pd–DNA structures observed by AFM and the voltammetric results suggest that the Pd$^{2+}$ interaction with DNA is very strong and stable.

Acknowledgements

Financial support from Fundação para a Ciência e Tecnologia (FCT), Post-Doctoral Grants SFRH/BPD/27087/2006 (A.M. Chiorcea-Paquim), and SFRH/BPD/36110/2007 (V.C. Diculescu.), Ph.D. Grants SFRH/BD/18914/2004 (O. Corduneanu) and SFRH/BD/27322/2006 (S.C.B. Oliveira), projecto PTDC/QUI/65255/2006 (co-financed by the European Community Fund FEDER), and CEMUC® (Research Unit 285), is gratefully acknowledged.
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